

Airway Closure after Antigen Challenge in Cynomolgus Monkeys: Effect of the Histamine H₁ Receptor Antagonist, Chlorpheniramine Maleate

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Key Words

Airway closure · Ascaris challenge · Chlorpheniramine maleate · Histamine H₁ receptor

Abstract

Background: Airway closure is frequently observed in human asthma. However, limited information exists on the factors that cause this condition. In this study, an allergic cynomolgus monkey model was used to characterize the condition of airway closure and assess the contribution of histamine H₁ receptors to this response. **Methods:** Oscillatory lung mechanics, arterial blood gases during ventilation on 100% O₂ and functional residual capacity (FRC) assessed by helium dilution were measured before and then 10 min and 24 h after Ascaris aerosol challenge in 12 male Ascaris-sensitive cynomolgus monkeys. The monkeys were pretreated with intravenous saline or chlorpheniramine maleate (0.3 mg/kg) in a randomized crossover design. **Results:** Ascaris challenge produced a large increase in airway resistance, an increase in lung tissue damping (G) that measures ventilation inhomogeneity in the lung, a reduction in arterial oxygen tension (PaO₂) during ventilation on 100% O₂ and a reduction in FRC. These effects were seen 10 min after the Ascaris challenge, but by 24 h, these parameters

had returned close to the baseline values. Chlorpheniramine maleate (0.3 mg/kg, i.v.) produced a 12-fold shift in the histamine bronchoconstrictor dose-response curve. Pretreatment of monkeys with chlorpheniramine maleate (0.3 mg/kg, i.v.) attenuated the increase in airway resistance induced by Ascaris challenge, but had only a small effect on the increase in G and the reductions in PaO₂ and FRC after antigen. **Conclusions:** These results demonstrate that airway closure occurs immediately after the antigen challenge in allergic cynomolgus monkeys and that histamine H₁ receptors contribute very minimally to this response.

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Introduction

Airway closure is a condition that is often seen in patients with obstructive lung diseases, such as asthma [1–9]. Furthermore, airway closure can occur in response to challenge with different bronchoconstrictor agents such as methacholine, exercise or cool dry air [5–8]. Depending upon the severity and duration of this condition, airway closure will have a number of adverse effects on pulmonary function and produce reduced lung volumes, impaired gas exchange and inhomogeneity of ventilation [7,

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8, 10]. Therefore, airway closure is an important factor involved in the pathophysiology of obstructive lung disorders such as asthma. However, little is known about the mechanisms producing this condition and even less is known about the pharmacological control of airway closure. In order to answer some of these questions, an animal model is needed that demonstrates some of the important features of human asthma, including the condition of airway closure.

The allergic cynomolgus monkey is often used in pulmonary research because it exhibits many of the characteristic features of human asthma such as eosinophilic pulmonary inflammation [11–14], reversible airflow obstruction [12, 13, 15, 16] and bronchial hyperresponsiveness [11, 13, 14, 17]. However, a detailed investigation into whether airway closure occurs in cynomolgus monkeys after antigen challenge has not been performed. Furthermore, many different allergic mediators are released into the lungs after antigen challenge [16], but the relative contribution of these different mediators to the development of airway closure has not been defined.

A variety of methods can be used to investigate the phenomenon of airway closure. Computed tomography, high-resolution computed tomography and hyperpolarized helium-3 magnetic resonance imaging have been used to detect the presence of air trapping and airway closure [2–5, 7, 8]. However, these techniques require specialized imaging equipment that is expensive to purchase and may involve the use of ionizing radiations. A simpler technique that has also been used to measure the regional inhomogeneity in the lung and separate the mechanical properties of the airway from that of the lung tissue component is the technique of forced oscillation [18–22]. The forced oscillation technique described by Hantos et al. [23] that models respiratory system impedance into Newtonian (or airway) resistance connected to a constant phase viscoelastic component measuring tissue damping (G) and tissue elastance (H) has been extensively used to evaluate lung mechanics. In this regard, the parameter G has been closely linked to ventilation inhomogeneities in the lung particularly during states of bronchoconstriction [23, 24]. Another technique that can be used to measure gas trapping and airway closure is to perform arterial blood gas analysis [10]. This method is particularly sensitive if it is performed during artificial ventilation on 100% O₂, because a reduction in PaO₂ under this condition can only be caused by increased right-to-left shunt [10]. Finally, the measurement of functional residual capacity (FRC) measured by helium dilution is another technique that can be used to assess the degree of airway

closure. This technique measures the dilution of helium gas after its inhalation from FRC and, as such, only measures the volume of air that is in free communication with the airways [25]. It does not measure the volume of air that is trapped beyond occluded airways. In this regard, FRC was found to be significantly lower in obese cynomolgus monkeys, and these lower FRCs correlated significantly with increased shunt fraction and reduced PaO₂ during 100% O₂ ventilation [25].

In the present study, oscillatory lung mechanics, including the parameter G, PaO₂ and FRC were assessed before and then 10 min and 24 h after antigen challenge in *Ascaris*-sensitive cynomolgus monkeys to determine if airway closure occurs in this species in response to allergen challenge. Histamine is an important mediator involved in the acute-phase allergic response in cynomolgus monkeys [13, 15, 16, 26]. Hence, the experiments were performed using the histamine H₁ receptor antagonist, chlorpheniramine maleate [27], to determine if histamine was involved in the airway closure resulting from antigen challenge in this species.

Materials and Methods

Animal Preparation

The experiments were performed on 12 male cynomolgus monkeys, ages 5–13 years, ranging in weight from 5 to 11 kg. These monkeys demonstrate a natural sensitivity to *Ascaris suum* and have been used previously in bronchoprovocation studies with aerosolized *A. suum* extract [14, 26, 28]. All experiments were sanctioned by the Animal Care and Use Committee of the Schering-Plough Research Institute, which is a facility approved by the American Association for Accreditation of Laboratory Animal Care.

The monkeys were tranquilized with intramuscular ketamine hydrochloride (10 mg/kg) and anesthesia was maintained throughout the experiments with intravenous propofol given as a bolus of 8 mg/kg and then infused at a rate of 0.04 mg/kg/min. A cuffed endotracheal tube was inserted into the trachea and the animals were artificially ventilated ($V_T = 8$ ml/kg, $f = 30$ breaths/min) on 100% O₂ using the Flexivent™ system (see below). Full cardiopulmonary monitoring was performed throughout the experiments, as previously described [18].

Respiratory system impedance was measured with a computer-driven primate ventilator (Flexivent, Scireq, Scientific Respiratory Equipment, Montreal, Quebec, Canada) that acquires data from the simultaneous measurement of piston volume displacement and intrapulmonary pressure. Respiratory system resistance (R_{rs}) and respiratory system elastance (E_{rs}) were measured using a single frequency (2 Hz) sinusoidal perturbation analyzed in the time domain. Respiratory system impedance was also measured over a range of frequencies (0.25–19.625 Hz), and the data fit to a model measuring Newtonian resistance (R_N) connected to a constant phase viscoelastic tissue component measuring tissue damping (G), tissue elastance (H) and the ratio of G/H termed lung hysteresivity (η) [23].

Ascaris Challenge

The monkeys were equilibrated for 10 min on the Flexivent respirator during ventilation with 100% O₂ and baseline measurements were obtained. An arterial blood sample was taken from the femoral artery under anerobic conditions and stored on ice until analyzed for PaO₂, PaCO₂, pH and HCO₃⁻ using an ABL500 blood gas analyzer (Radiometer American Inc., Westlake, Ohio) and arterial oxygen saturation (SaO₂) was measured using a radiometer OSM 3 co-oximeter. Barometric pressure was recorded daily. Venous admixture was calculated from the modified shunt equation, as previously described [25]. After collection of the arterial blood sample, FRC was measured using the method of helium dilution, as previously described [25]. Briefly, the endotracheal tube was disconnected from the Flexivent respirator, a gas syringe containing 44 ml of a 10% helium/90% nitrogen mixture was attached to the end of the endotracheal tube and the gas mixture was rapidly equilibrated with the air in the lungs by expelling from the syringe and retrieving it several times. The concentration of helium in the equilibrated gas was determined using a mass spectrometer and the FRC was calculated using the standard helium dilution equation [25].

The monkeys were then challenged with 15 breaths of aerosolized *A. suum* at a concentration which is historically known to trigger an acute-phase bronchospasm [14, 26, 28]. The concentration of *A. suum* extract differed for each monkey and ranged from 1:100,000 to 1:1% w/v. The aerosols were produced with an ultrasonic nebulizer (DeVilbiss, Ultra-Neb 99) and delivered directly into the endotracheal tube with a Harvard respirator at volumes identical to that used during artificial ventilation on the Flexivent. Respiratory mechanics were measured for approximately 10 min after the *A. suum* challenge at which time an arterial blood sample was withdrawn and the FRC was measured.

The monkeys were then returned to their cages and 24 h later, they were reanesthetized and artificially ventilated on 100% O₂ with the Flexivent respirator. Ten minutes later, respiratory mechanics were measured, an arterial blood sample was withdrawn and the FRC maneuver was performed.

Effect of Chlorpheniramine Maleate

The 12 monkeys were given intravenous injection of either chlorpheniramine maleate (0.3 mg/kg) or isotonic saline (0.1 ml/kg) 10 min before the challenge with the aerosolized *Ascaris*. The dose of chlorpheniramine maleate (0.3 mg/kg, i.v.) was chosen from experiments involving histamine bronchoprovocation as a dose that produced a greater than 10-fold shift in the histamine bronchoconstrictor dose-response curve (see 'Results'). In these histamine bronchoprovocation studies, the monkeys were anesthetized, instrumented for the measurement of respiratory mechanics and ventilated on 100% O₂. Baseline lung mechanics were first obtained followed by intravenous injection of chlorpheniramine maleate (0.03–0.3 mg/kg) or saline (0.1 ml/kg). Ten minutes after the chlorpheniramine maleate or saline injection, the monkeys were challenged with five consecutive breaths of aerosolized histamine ranging in concentrations from 0.03 to 10 mg/ml and delivered directly to the endotracheal tube with a Harvard respirator. Pulmonary mechanics were measured after each dose of histamine until Rrs had doubled from the baseline values. The data were expressed as the provocative concentration of histamine increasing Rrs by 50% above the baseline values (PC₅₀, mg/ml).

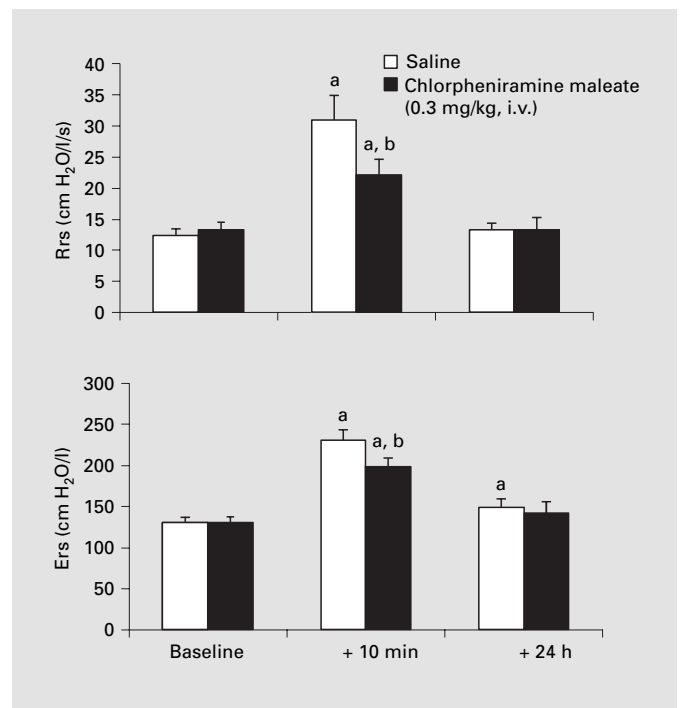


Fig. 1. Effect of chlorpheniramine maleate on Rrs and Ers before (baseline), and 10 min and 24 h after *Ascaris* challenge. ^a $p \leq 0.05$ compared with baseline values before *Ascaris* challenge. ^b $p \leq 0.05$ compared with saline treatment.

Statistical Analysis

All studies were performed using a randomized crossover experimental design following treatment with either intravenous saline or chlorpheniramine maleate. The monkeys were rested for at least 2 weeks between studies. A paired t test was used to determine statistically significant effects of *Ascaris* challenge from baseline values and to determine significant effects of the chlorpheniramine maleate treatment compared with saline. The correlation coefficient (r) was calculated using a Pearson's correlation method, and regression analysis was used to determine whether the correlations were statistically significant. All statistical analyses were performed using Statview (SAS Institute Inc., Cary, N.C., USA). p values < 0.05 were taken as significant.

Results

Ascaris challenge produced a highly significant increase in Rrs and Ers immediately after the antigen challenge (fig. 1). However, by 24 h after the antigen challenge, both Rrs and Ers had returned close to the baseline values although Ers was still significantly ($p < 0.05$) higher than the baseline. Similar results were obtained for R_N , G , H and η that increased significantly by 10 min after the

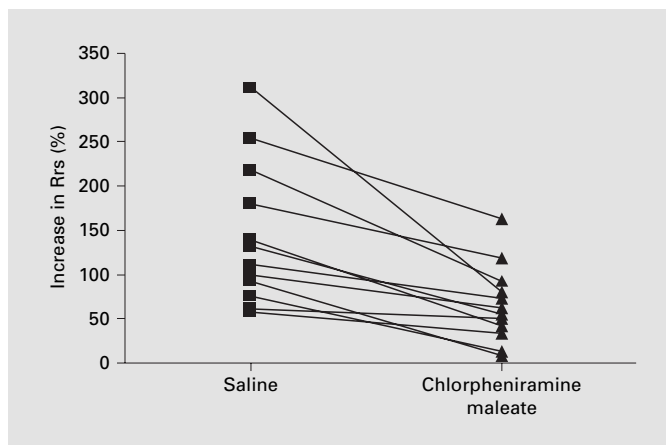


Fig. 2. Inhibition of the *Ascaris*-induced increase in Rrs by chlorpheniramine maleate. Values are the percentage increases in Rrs induced by *Ascaris* in each of the 12 monkeys.

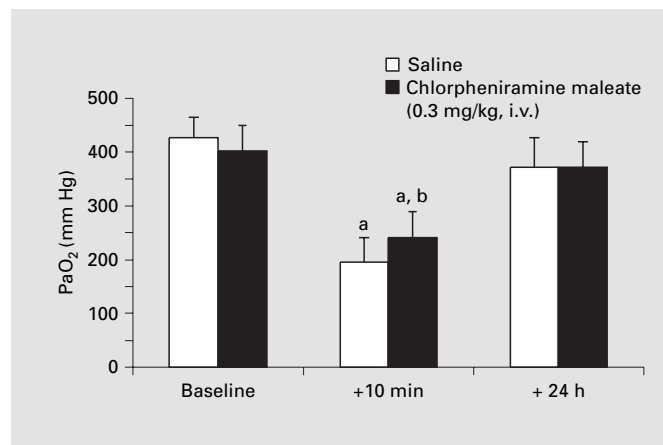


Fig. 3. Effect of chlorpheniramine maleate on PaO_2 before (baseline), and 10 min and 24 h after *Ascaris* challenge. ^a $p \leq 0.05$ compared with baseline values before *Ascaris* challenge. ^b $p \leq 0.05$ compared with saline treatment.

Table 1. Effect of chlorpheniramine maleate on changes in respiratory mechanics after *Ascaris* challenge

Parameter	Saline (i.v.)			Chlorpheniramine maleate (0.3 mg/kg, i.v.)		
	baseline	10 min	24 h	baseline	10 min	24 h
R_N , cm $\text{H}_2\text{O}/\text{ml}/\text{s}$	5.0 ± 0.6	7.5 ± 1.2^a	5.4 ± 0.5	5.2 ± 0.4	6.2 ± 0.8	5.3 ± 0.6
G, cm $\text{H}_2\text{O}/\text{ml}$	51 ± 3	92 ± 6^a	52 ± 3	48 ± 3	$76 \pm 6^{a,b}$	53 ± 4
H, cm $\text{H}_2\text{O}/\text{ml}$	213 ± 11	264 ± 11^a	227 ± 14	212 ± 9	249 ± 14^a	213 ± 12
η	0.24 ± 0.02	0.35 ± 0.02^a	0.23 ± 0.01	0.23 ± 0.01	$0.31 \pm 0.02^{a,b}$	0.25 ± 0.01

Values are means $\pm$ SEM ($n = 12$ per treatment) before (baseline), and 10 min and 24 h after *Ascaris* challenge.

^a $p < 0.05$ compared with baseline.

^b $p < 0.05$ compared with saline treatment.

Ascaris challenge but had returned close to the baseline values by 24 h (table 1). Pretreatment of the monkeys with chlorpheniramine maleate (0.3 mg/kg, i.v.) reduced the increase in Rrs induced by *Ascaris* in each of the 12 monkeys studied (fig. 2). Chlorpheniramine maleate also inhibited the *Ascaris*-induced increase in Ers, although the magnitude of this inhibition was not as pronounced as the inhibition of Rrs (fig. 1). Similar results were obtained for R_N , G, H and η , with the greatest inhibition by chlorpheniramine maleate seen on the *Ascaris*-induced increase in R_N (62% inhibition) compared with the inhibition seen on G (28% inhibition), H (29% inhibition) and η (24% inhibition).

Ascaris challenge produced a significant reduction in PaO_2 10 min after the challenge, but by 24 h, the PaO_2 returned close to the baseline values (fig. 3). The reduction in PaO_2 was associated with an increase in venous admixture which increased from baseline values of $14 \pm 2\%$ before the *Ascaris* challenge to $29 \pm 3\%$ 10 min after the *Ascaris* challenge. There were no significant changes in PaCO_2 , pH or HCO_3^- measured 10 min after the *Ascaris* challenge (data not shown). Pretreatment of the monkeys with chlorpheniramine maleate (0.3 mg/kg, i.v.) had only a small inhibitory effect on the acute fall in PaO_2 observed 10 min after the *Ascaris* challenge (fig. 3). There was also a significant reduction in FRC 10 min after the *Ascaris* challenge (fig. 4). However, the FRC had returned

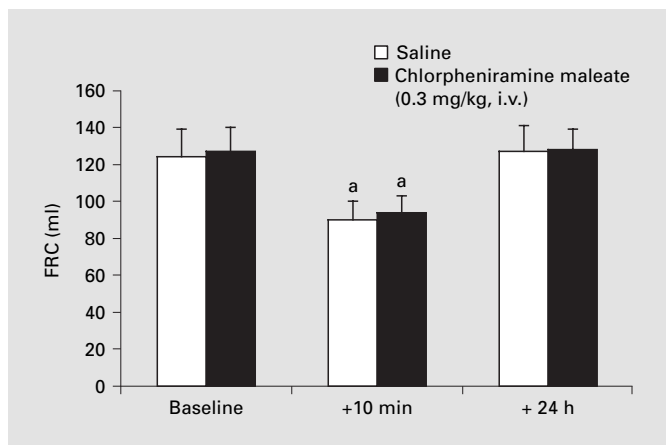


Fig. 4. Effect of chlorpheniramine maleate on FRC before (baseline), and 10 min and 24 h after *Ascaris* challenge. ^a $p \leq 0.05$ compared with baseline values before *Ascaris* challenge.

close to the baseline values by 24 h after the challenge. Chlorpheniramine maleate (0.3 mg/kg, i.v.) had no effect on the acute reduction in FRC induced by the *Ascaris* challenge.

The data in figure 5 show the correlations between G and PaO₂ and FRC and PaO₂ in the 12 monkeys (treated with intravenous saline and chlorpheniramine maleate) 10 min after the *Ascaris* challenge. There was a highly significant ($p = 0.0001$) negative correlation ($r = 0.70$) between G and PaO₂. Furthermore, a statistically significant ($p = 0.02$) positive correlation ($r = 0.48$) was also found between FRC and PaO₂.

The bronchoconstrictor response to histamine was dose-dependently (0.03–0.3 mg/kg, i.v.) inhibited by chlorpheniramine maleate (table 2). Doses of 0.03, 0.1 and 0.3 mg/kg of intravenous chlorpheniramine maleate given 10 min before the histamine challenge shifted the bronchoconstrictor dose-response curve by 3.5-, 7- and 12-fold, respectively.

Discussion

The goal of this study was to determine if airway closure occurred after antigen challenge to *Ascaris*-sensitive cynomolgus monkeys. Furthermore, experiments were performed with the histamine H₁ receptor antagonist, chlorpheniramine maleate, to determine the role of histamine H₁ receptors on the airway closure caused by the antigen challenge. The results show that immediately

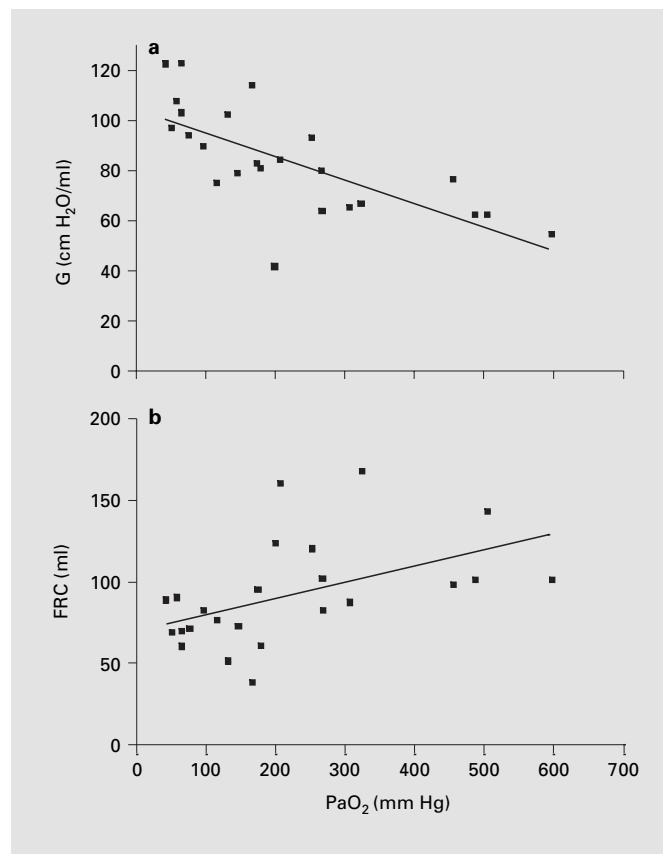


Fig. 5. Correlations between PaO₂ and lung tissue damping (G) (**a**) and FRC (**b**) 10 min after *Ascaris* challenge.

Table 2. Dose-response effect of chlorpheniramine maleate on the bronchoconstrictor response to histamine

Treatment	Dose mg/kg i.v.	n	PC ₅₀ mg/ml ^a	Fold shift
Saline	0	3	0.09 ± 0.07	
Chlorpheniramine maleate	0.03	3	0.32 ± 0.14	3.5
Saline	0	3	0.52 ± 0.23	
Chlorpheniramine maleate	0.1	3	3.59 ± 1.71	7
Saline	0	6	0.27 ± 0.11	
Chlorpheniramine maleate	0.3	6	3.23 ± 1.05 ^b	12

Values are means ± SEM measured 10 min after saline or chlorpheniramine maleate.

^a PC₅₀ – provocative concentration of histamine, increasing Rrs by 50% above baseline.

^b $p \leq 0.05$ compared with saline.

after the antigen challenge, there was an increase in tissue damping, a parameter closely linked to ventilation inhomogeneity in the distal lung [23, 24], a reduction in PaO₂ during 100% O₂ ventilation, denoting the presence of a large right-to-left shunt [10], and a reduction in FRC. These results demonstrate that airway closure occurs immediately after the antigen challenge. However, the airway closure did not persist beyond 24 h as these reduced lung functions and impaired gas exchange had returned close to the baseline values by 24 h after the antigen challenge. Our results with chlorpheniramine maleate also show that histamine H₁ receptors contribute only minimally to the airway closure as the increase in G, reduction in PaO₂ and FRC in response to the *Ascaris* challenge were only marginally improved after chlorpheniramine maleate treatment.

The techniques that we used to assess the presence of airway closure included the measurement of forced oscillation, arterial blood gas analysis during ventilation on 100% O₂ and FRC measured by helium dilution. Forced oscillation is often used to measure airway and lung tissue impedance after bronchoprovocation [19, 20, 23, 24, 29] and has been used in cynomolgus monkeys to assess the effects of antigen challenge [30–32]. In this study, we used the method described by Hantos et al. [23] that models respiratory system impedance into Newtonian (airway) resistance and a constant phase viscoelastic component measuring tissue damping (G), tissue elastance (H) and the ratio of G/H termed lung hysteresivity (η). The parameter G is strongly influenced by any change in ventilation inhomogeneity in the lung, particularly during states of bronchoconstriction [23, 24]. Given the fact that most of the increase in respiratory system impedance after antigen challenge in cynomolgus monkeys is due to an increase in airway resistance [31, 32], the increase in G that occurred after antigen is probably the result of ventilation inhomogeneity in the distal lung rather than a true increase in tissue resistance. Factors that increase ventilation inhomogeneities would include airway closure [1, 7, 8, 23, 24].

Airway closure was also assessed by measuring arterial blood gases during ventilation on 100% O₂. A reduction in PaO₂ under these conditions denotes the presence of a large right-to-left shunt and distinguishes the cause of the arterial hypoxemia from that caused by impaired diffusion or ventilation/perfusion mismatch [10]. Arterial hypoxemia after antigen challenge has been previously described in cynomolgus monkeys [25, 33], but the results in our study are the first to show that airway closure and increased right-to-left shunt contribute to this response.

There was also a reduction in FRC immediately after the antigen challenge. As the helium dilution technique measures only the volume of gas freely communicating with the airways, it will not measure the fraction of gas that is trapped beyond distal occluded airways. Thus, the reduction in FRC immediately after the antigen challenge may include the phenomenon of airway closure, although we cannot completely rule out a possible contribution from the bronchoconstrictor effect of the antigen challenge to this response. However, a significant correlation was found between FRC and PaO₂ following the antigen challenge. Furthermore, a significant correlation between G and PaO₂ was also demonstrated in the monkeys immediately after antigen. Therefore, a close interrelationship exists between the parameters of lung tissue damping, FRC and reduced arterial blood oxygenation following the antigen challenge that we interpret as evidence of airway closure.

Although there was clear evidence of airway closure immediately after the antigen challenge, this condition had almost fully resolved by 24 h later. The only parameter that still showed slight elevation at this time was Ers, but every other parameter, including the fall in PaO₂, had returned to the baseline values. In their colony of allergic cynomolgus monkeys, Gundel et al. [12] also reported that Rrs returned to the baseline values by 24 h after the antigen challenge. However, no assessment of airway closure was made in Gundel's study [12]. The fact that baseline lung functions, including airway closure, had almost fully resolved by 24 h after antigen challenge has important implications to the role of pulmonary inflammation and lung function in this model. Previous studies have demonstrated a large influx of eosinophils into the airways of monkeys by 24 h after antigen challenge [11, 13, 14, 17, 28]. As we found virtually no change in the baseline lung functions by 24 h after antigen challenge, it would appear that airway eosinophilia has little impact on baseline lung functions in this model.

Histamine is an important mediator of acute allergic reactions in monkeys [13, 15, 16, 26, 33] and is a potent constrictor of airway smooth muscle in this species [34]. Previous studies in allergic cynomolgus monkeys have demonstrated a partial inhibition of the acute-phase bronchospasm after treatment of the monkeys with histamine H₁ receptor antagonists [13, 15, 26]. However, in each of these previous studies, the bronchoconstrictor response to the antigen was assessed using the measurement of airway resistance, and no assessment was done to evaluate effects in the distal lung such as airway closure [13, 15, 26]. The results in our study confirm that a partial inhibitory effect

of histamine H₁ receptor antagonists is seen on the acute-phase bronchospasm as we found significant effects of chlorpheniramine maleate on the associated increase in airway resistance after antigen challenge. However, chlorpheniramine maleate had only a very small effect on the increase in G, reduction in PaO₂ and reduction in FRC after antigen. Therefore, histamine appears to have only a very small effect on the airway closure occurring immediately after antigen challenge, at least by acting through stimulation of the histamine H₁ receptor. Increasing the dose of chlorpheniramine maleate to 1 mg/kg intravenously produced no additional inhibition of the acute-phase bronchospasm and airway closure above that seen with the 0.3-mg/kg dose (unpublished observations). Furthermore, the dose of chlorpheniramine maleate that we used for this evaluation, i.e. 0.3 mg/kg, produced a 12-fold shift in the bronchoconstrictor dose-response curve which is a response that is mediated through activation of histamine H₁ receptors [13, 26, 34]. These results indicate that

in the allergic cynomolgus monkey, the effect of histamine is seen predominantly on the conducting airway with much smaller effects in the distal lung.

In conclusion, this study used the parameters of lung tissue damping, arterial PO₂ and FRC to determine whether airway closure occurs in cynomolgus monkeys following an antigen challenge. The results demonstrate that airway closure occurs immediately after the antigen bronchoprovocation, but this condition fully resolves by 24 h after the antigen challenge. Using chlorpheniramine maleate to evaluate the contribution of histamine H₁ receptors to this response, it was found that histamine plays only a minor role in the acute airway closure occurring immediately after the antigen challenge. Although the important allergic mediators producing the airway closure still need to be identified, the allergen-challenged cynomolgus monkeys appear to be useful models to investigate this phenomenon.

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