

Respiratory responses to *Ascaris* antigen in rhesus and cynomolgus monkeys

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Responses to Ascaris antigen were evaluated in rhesus and cynomolgus monkeys. Of 19 cynomolgus monkeys tested, 15 were found to have cutaneous reactivity to Ascaris: 13 of these responded to Ascaris aerosols with changes in respiratory function including an increase in respiration rate, decrease in tidal volume and peak expiratory flow rate, decrease in dynamic compliance, and an increase in resistance. Four of the six rhesus monkeys studied with cutaneous reactivity to Ascaris responded to Ascaris aerosols: the changes in respiratory function observed after the Ascaris challenge in these monkeys were similar to those observed in the cynomolgus monkeys. Responses to repetitive Ascaris challenges were obtained in monkeys of both species with a recovery period of 30 to 60 min between challenges. In monkeys of both species that reacted to Ascaris aerosols, blood pressure was elevated transiently; changes in heart rate were variable. Changes in cardiovascular measurements were not observed in monkeys that did not respond to Ascaris aerosols. Thus, it appears that the cynomolgus monkey responds to an aerosol Ascaris challenge in a manner similar to the rhesus monkey and is an additional suitable model for the study of allergic respiratory responses.

In an attempt to characterize the mechanisms involved in human asthma, various animal models have been employed.¹⁻⁴ In one type of human asthma, an asthmatic attack is provoked by antigen inhalation, and the antigen then reacts with reaginic antibodies fixed to lung mast cells with subsequent mediator release and bronchoconstriction. The presence of reagin-like antibodies in animals immune to helminth parasites is well known and has been studied in rats infected with *Nippostrongylus brasiliensis*,^{5, 6} rabbits infected with *Toxocara*, *Toxascaris*, and *Ascaris*,⁷ and monkeys infected with *Ascaris*.^{2, 3} In all cases, the reaginic antibody appears to be stimulated by only a living infection, and immunity persists for a long period of time. In the rhesus monkey (*Macaca mulatta*), the changes in ventilatory function after an *Ascaris* challenge, i.e., an increase in lung resistance and an apparent narrowing of central and peripheral airways, have been shown to be similar to those occurring in human asthma.⁸ Furthermore, the induced responses are reproducible over an extended length of

time,^{9, 10} and responses to two challenges of *Ascaris* in the same experimental period can be obtained,¹¹ thus constituting an in vivo test system for reagin-mediated respiratory responses.¹² However, a major limitation of this model is that animals with respiratory reactivity to *Ascaris* constitute only a small portion of unselected animals, estimated at less than 5%.¹² The purpose of the present study was to investigate the reactivity of another species of monkey, the cynomolgus monkey (*Macaca fascicularis*), to *Ascaris* antigen and to compare respiratory responses to *Ascaris* in rhesus and cynomolgus monkeys.

MATERIALS AND METHODS

Animals

Male and female cynomolgus (*Macaca fascicularis*) and rhesus (*Macaca mulatta*) monkeys obtained from Primate Imports were used in these studies. They were in good health, free of parasite infestation, and all had negative tests to tuberculinoprotein.

Ascaris antigen

An extract of pig *Ascaris* (Asc) was obtained from Greer Laboratories (Lenoir, N. C.) containing 140,000 to 194,000 PNU/ml in Coca's solution (NaCl 5.0 gm/L; NaHCO₃ 5.4 gm/L; phenol 4.0 gm/L). Dilutions were made in sterile water for aerosol challenges and were prepared freshly every 2 wk.

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TABLE I. Baseline pulmonary function measurements in pentobarbital-anesthetized rhesus and cynomolgus monkeys

Monkey Type	Sex	Weight (kg)	f (per min)	PEFR (ml/sec)	V _T (ml)	R _L * (cm H ₂ O/LPS)	C _{DYN} (ml/cm H ₂ O)
Rhesus	Male (3)	9.2 ± 1.1	25.4 ± 5.2	116.6 ± 11.6	56.8 ± 4.2	16.7 ± 0.4	37.6 ± 7.4
Rhesus	Female (4)	5.4 ± 1.2	23.0 ± 2.6	65.9 ± 6.0	41.7 ± 3.9	33.0 ± 4.2	25.2 ± 3.1
Cynomolgus	Male (15)	5.2 ± 0.9	28.9 ± 2.0	82.0 ± 3.6	34.6 ± 2.2	23.6 ± 1.5	20.1 ± 2.0
Cynomolgus	Female (4)	2.7 ± 0.4	26.5 ± 2.9	44.4 ± 5.7	19.9 ± 1.9	32.6 ± 2.5	18.3 ± 5.6

Values are means ± SE for the number of monkeys in parentheses.

*Includes resistance of apparatus.

Cutaneous testing

Animals were anesthetized with ketamine (10 mg/kg intramuscularly), and a volume of 0.5 ml/kg of a 2% coomassie blue (Imperial Chemical Industries, Ltd., England) or a 0.5% Evans blue (Eastman Kodak Co., Rochester, N. Y.) dye solution was injected intravenously a few minutes before skin tests were conducted. One-tenth-milliliter volumes of sterile 0.45% saline, 1:10 dilution of Coca's solution, and serial tenfold dilutions (in saline) of Asc ranging from 1:10 to 1:100,000 were injected intradermally. In some animals additional skin tests using 0.0001% to 1.0% ovalbumin (OV) were performed to check for reactivity to another protein. Responses were recorded as follows: S, strong response (large, intense blue wheal); W, weak response (small, light blue wheal); and N, negative response (no bluing observed).

Lung and cardiovascular function recording

Monkeys were anesthetized with sodium pentobarbital either intramuscularly or intravenously and an endotracheal tube (i.d. 4.5 to 5.5 mm, length 22 to 23 cm including connector, Willy R sch, GmbH and Co. KG, West Germany) was inserted a few centimeters into the trachea. A heated Fleisch size 00 pneumotachograph (Dynasciences Medical Products, Blue Bell, Pa.) connected to a Validyne differential pressure transducer (± 20 cm H₂O, Validyne Engineering Corp., Northridge, Calif.) was used to measure respiratory flow. For the measurement of transpulmonary pressure, a latex balloon (2 × 10 × 150 mm, Dynasciences Medical Products) was inserted in the esophagus to the level of the nipple line and connected with polyethylene tubing to one end of a Validyne differential pressure transducer (± 2 cm H₂O) with the other end open to the atmosphere. A Buxco Model 6 Pulmonary Mechanics Computer (Buxco Electronics Inc., Sharon, Conn.) was used to compute dynamic compliance (C_{dyn}) and resistance (R_L) from the input signals of flow (V) and transpulmonary pressure (P_L). In computing R_L, the resistance of the entire apparatus was included, which was 12 cm H₂O/LPS at 0.083 LPS using a 5.0-mm (i.d.) endotracheal tube. Tidal volume (V_T) was calculated by integrating the V signal. Needle electrodes were inserted subcutaneously in the upper right arm, lower left leg, and chest for the measurement of a lead II electrocardiogram (ECG). In some experiments, a blood pressure (BP) measurement was obtained by short-term arterial catheterization of the tibial artery using a 20-gauge

Abbocath-T catheter (Abbott Hospital Products, Chicago) connected by tubing to a Statham pressure transducer (Model P23ID). Measurements of V, P_L, V_T, C_{dyn}, R_L, BP, respiration rate (f), and the lead II ECG were continuously recorded on a Gould Brush 480 recorder (Gould Inc., Instrument Systems Div., Cleveland, Ohio). Calculated values from the pulmonary computer for peak expiratory flow rate (PEFR), P_L, V_T, C_{dyn}, R_L, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure, and heart rate (HR), were converted to digital signals by a DL-12 data logger (Buxco Electronics, Inc.) and then printed on a Texas Instruments teleprinter. Baseline pulmonary measurements for the animals used in these studies are shown in Table I.

Aerosol challenges

Aerosols containing Asc were generated using a DeVilbiss 35B nebulizer (The DeVilbiss Co., Somerset, Pa.) and delivered to the animal directly through the endotracheal tube while the animal was breathing normally. A 1:5 dilution of Asc was initially administered to animals for a maximum of 5 min to determine if a respiratory response to Asc could be elicited. An animal was considered a positive respiratory responder if at least a 75% increase in f and a 25% decrease in V_T were observed after the Asc challenge. Animals in which a respiratory response was not evident within 30 min after a 5-min Asc exposure were considered negative responders. If an animal began to react during the Asc exposure, the aerosol was stopped. In other experiments, repeated brief challenges using lower concentrations of Asc (1:10 or 1:20 dilution of Asc) were administered to some animals to determine if more than one response could be induced in the same experimental period, and if these responses were of similar magnitude. The concentration of Asc used for each animal in these studies was determined to induce changes in respiratory measurements that would return close to baseline values within to 30 to 60 min. A 3-wk interval was allowed before repeating an experiment with the same animal.

RESULTS

Cutaneous reactivity to Asc

Of the 19 cynomolgus monkeys tested for skin reactivity to Asc, 74% (15/19) demonstrated strong cutaneous responses to Asc (Table II). For compara-

TABLE II. Skin and respiratory reactivity to *Ascaris* antigen in cynomolgus and rhesus monkeys

Animal	Skin reactivity*					Respiratory reactivity†
	1:10	1:100	1:1,000	1:10,000	1:100,000	
Cynomolgus						
A	N	N	N	N	N	Negative
B	N	N	N	N	N	Negative
C	N	N	N	N	N	Negative
D	W	W	W	N	N	Negative
E	S	S	S	W	N	Negative
F	S	S	S	S	W	Negative
G	S	S	N	N	N	Positive
H	S	S	S	W	W	Positive
I	S	S	S	W	N	Positive
J	S	S	S	W	N	Positive
K	S	S	S	W	N	Positive
L	S	S	S	W	N	Positive
M	S	S	S	W	W	Positive
N	S	S	S	S	W	Positive
O	S	S	S	S	W	Positive
P	S	S	S	S	W	Positive
Q	S	S	S	S	W	Positive
R	S	S	S	S	S	Positive
S	S	S	S	S	S	Positive
Rhesus						
A	N	N	N	N	N	Negative
B	S	S	S	S	S	Negative
C	S	W	W	W	W	Positive
D	S	W	W	W	W	Positive
E	S	W	W	W	W	Positive
F	S	S	S	S	S	Positive
G	S	S	S	S	S	Positive

*Cutaneous reaction to dilutions of the *Ascaris* antigen extract containing 140,000 to 194,000 PNU/ml. Reactions were scored as follows:

S: strong response; W: weak response; N: no response.

†See text for explanation of negative and positive respiratory reactivity.

tive purposes, results of skin tests using six rhesus monkeys that had been previously determined to have skin reactivity to Asc (B through G) and one (A) that was shown to be nonreactive are also shown in Table II. No monkey of either species reacted to saline or Coca's solution. In most animals, cutaneous reactivity was indicative of respiratory reactivity to Asc, although two cynomolgus monkeys (E and F) and one rhesus monkey (B) with strong cutaneous reactivity did not respond to Asc aerosols. No monkey of either species with negative skin reactivity displayed respiratory reactivity to Asc.

In the animals tested for skin reactivity to Asc, a weak response to OV was observed only with an intradermal injection of 1.0% OV; tenfold lower concentrations of OV did not evoke skin responses. No respiratory response was observed when these monkeys (including one with strong respiratory reactivity to Asc) were administered an aerosol containing 2 mg/ml OV for 4 min.

Changes in respiratory function

All monkeys were first administered aerosols of water and a 1:5 dilution of Coca's solution for 5 min to test for a nonspecific reaction to the aerosol. No monkey reacted in a manner indicative of respiratory irritation after administration of these aerosols.

Figs. 1 and 2 depict the effects of an aerosol of a 1:5 dilution of Asc given for 5 min to two monkeys with negative cutaneous reactivity to Asc. Responses were measured for 25 min. In both monkeys, changes from baseline respiratory measurements were no greater than those normally observed after administration of a water aerosol. Monkeys with negative cutaneous responses to Asc were also tested 3 wk later with Asc aerosols to determine if respiratory reactivity would be induced; however, all of these monkeys remained consistently negative.

Typical responses to 1-min challenge with a 1:10 dilution of Asc in Asc-sensitive rhesus (D, E) and cynomolgus (L, N) monkeys are shown in Fig. 3. In

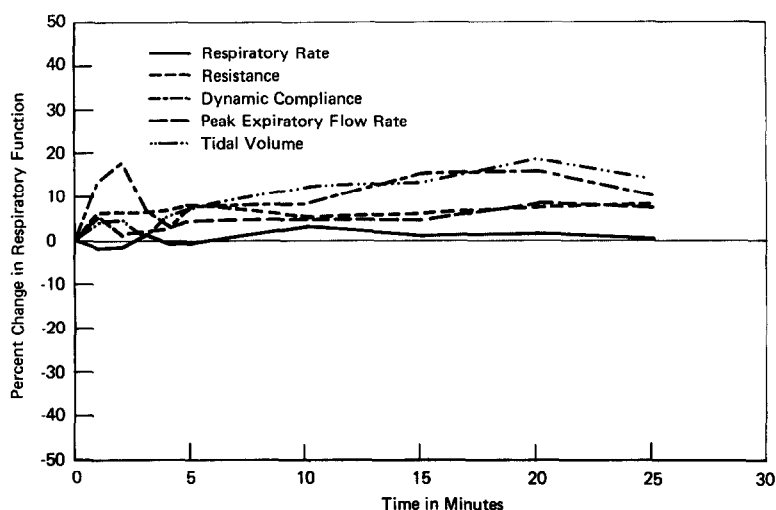


FIG. 1. Respiratory responses to Asc in a rhesus monkey (A) with negative cutaneous reactivity to Asc. Time 0 is the end of administration of an aerosol containing a 1:5 dilution of Asc for 5 min.

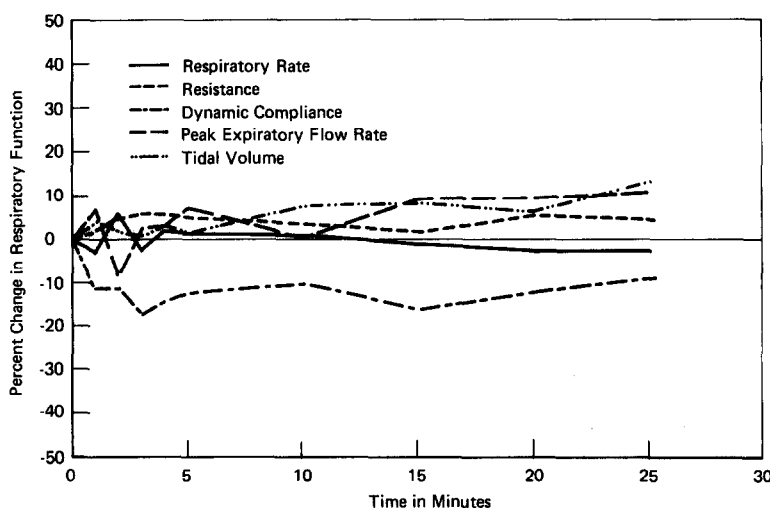


FIG. 2. Respiratory responses to Asc in a cynomolgus monkey (C) with negative cutaneous reactivity to Asc. Time 0 is the end of administration of an aerosol containing a 1:5 dilution of Asc for 5 min.

all four monkeys, responses were rapid in onset, usually occurring within 1 to 5 min after the start of the aerosol. After the Asc challenge, f increased nearly 100% to 250% from control values, V_T decreased 40% to 60%, and PEFR decreased 20% to 50% in these monkeys. The maximum percentage decrease from control values of C_{dyn} ranged from 30% to 60%. Changes in R_L following the Asc challenge were variable, ranging from only an 8% increase in rhesus monkey D to nearly a 50% increase in cynomolgus monkey N. Although the magnitude of the Asc-induced changes in respiratory function differs among animals, the general pattern of changes is similar in both species.

Responses to a few brief (1- or 2-min) repetitive

Asc challenges with a recovery period of 30 to 60 min between challenges were obtained. Figs. 4 and 5 depict the results of repetitive Asc challenges in a rhesus (F, Fig. 4) and a cynomolgus (P, Fig. 5) monkey. Three challenges of Asc were administered to these monkeys. As can be seen, the response to the initial challenge of Asc was greater in magnitude than to subsequent challenges. The diminished response to a subsequent challenge of Asc was observed in all of the rhesus monkeys in which repeated challenges of Asc were administered (D, F, and G) and some of the cynomolgus monkeys (H, L, and S). In a few cynomolgus monkeys (G, K, and Q) a larger increase in f from baseline values was observed after the second challenge of Asc than after the first.

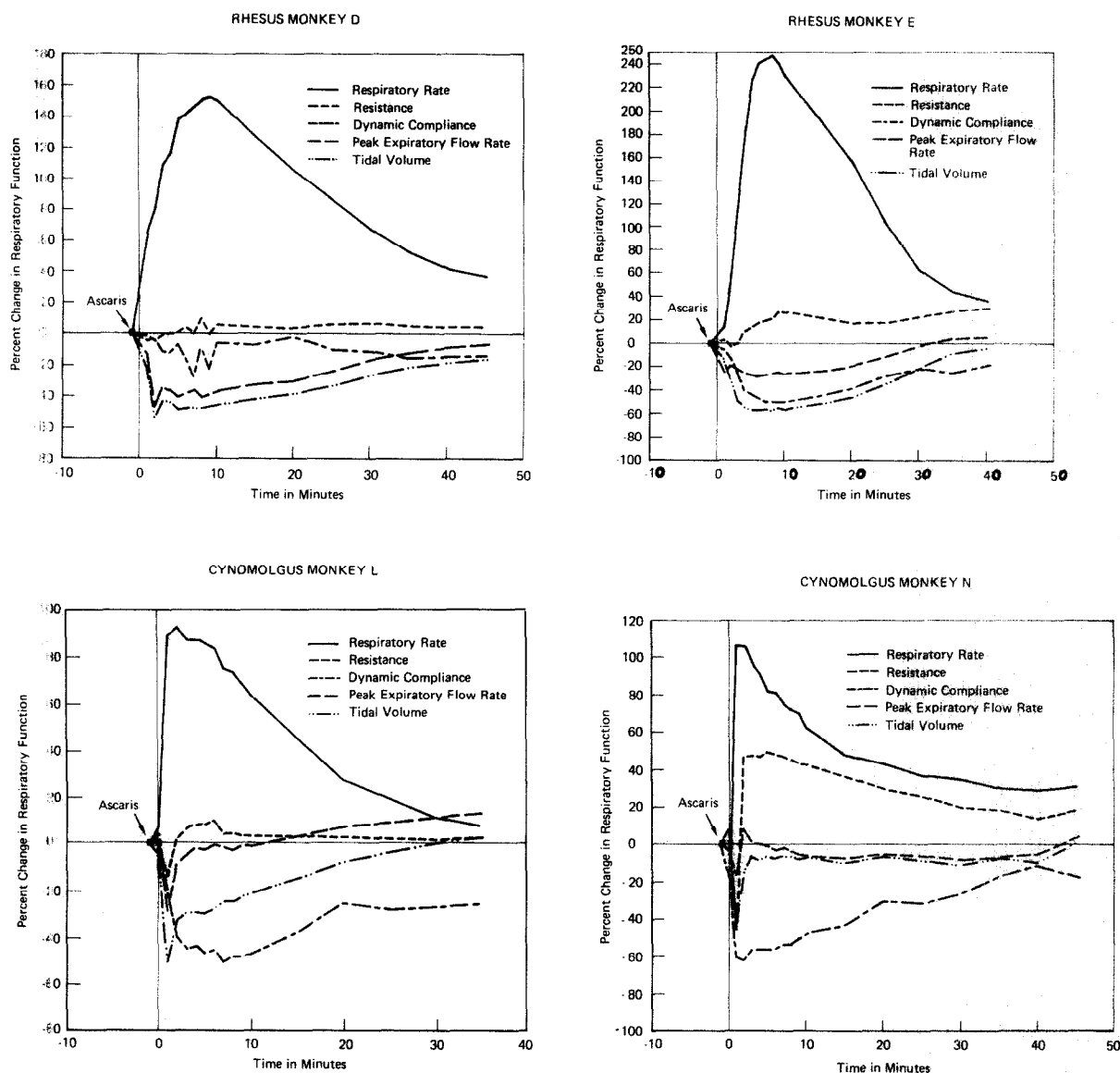


FIG. 3. Respiratory responses to Asc in Asc-sensitive rhesus (D, E) and cynomolgus (L, N) monkeys. The Asc challenge was 1 min in duration with a 1:10 dilution of Asc.

Changes in cardiovascular function

Measurements of HR, SBP, and DBP before and after an Asc challenge in rhesus and cynomolgus monkeys that responded to Asc are shown in Table III. In all animals, BP was elevated after an Asc aerosol, although changes in HR were variable. These measurements usually returned to control levels within 1 hr after the Asc administration. The peak effects on HR, SBP, and DBP were observed at approximately the same time as were the peak effects on respiratory measurements. In monkeys that did not respond to an Asc challenge, cardiovascular measurements were not affected after the Asc challenge. No abnormalities in the ECG pattern were observed

after Asc administration in monkeys with positive or no responses to Asc.

DISCUSSION

The rhesus and cynomolgus monkey are closely related species of the genus *Macaca*. Furthermore, pulmonary parameters, respiratory blood gas, and pH values in the cynomolgus and rhesus monkey have been shown to be similar.¹³⁻¹⁵ The *Ascaris*-sensitive rhesus monkey that gives persistent and reproducible respiratory responsiveness to Asc has been considered to be the closest analog in animals to human extrinsic or reagin-mediated asthma.¹⁶ However, the paucity of rhesus monkeys that react to Asc aerosols has been a

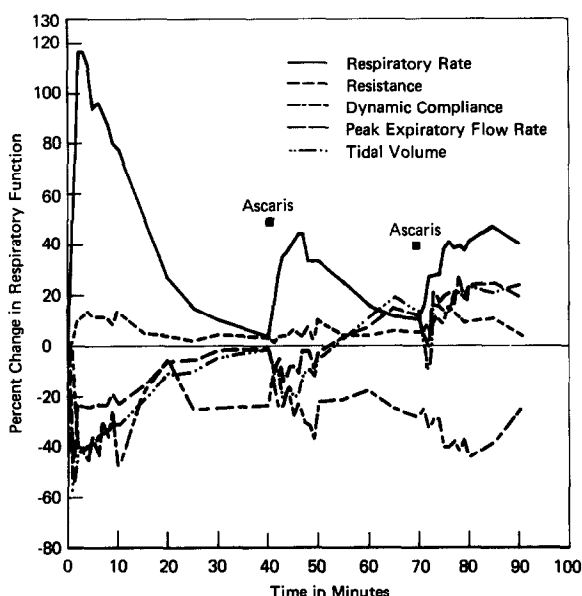


FIG. 4. Respiratory responses to repetitive aerosol challenges of a 1:20 dilution of Asc for a 1-min period in rhesus monkey F.

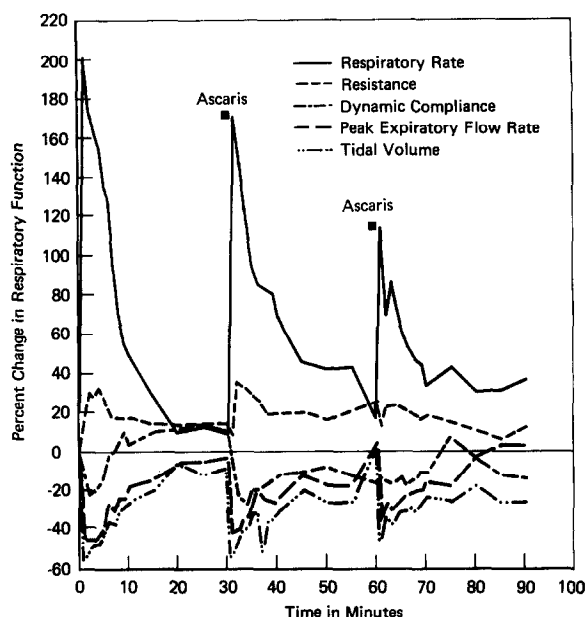


FIG. 5. Respiratory responses to repetitive aerosol challenges of a 1:20 dilution of Asc for a 1-min period in cynomolgus monkey P.

TABLE III. Effects of *Ascaris* aerosol* on blood pressure and heart rate in rhesus and cynomolgus monkeys

Animal	Control†		Ascaris‡	
	BP (mm Hg)	HR (beats/min)	BP (mm Hg)	HR (beats/min)
Rhesus				
D	84/38	141	108/53	143
E	110/59	137	118/62	140
F	76/48	116	124/64	115
G	81/45	152	95/52	175
Cynomolgus				
G	102/54	143	146/92	133
J	82/33	140	137/64	154
O	106/60	143	111/78	189
Q	95/51	150	132/70	161
R	82/34	131	93/38	157

*Asc aerosol challenges were 1 to 5 min in duration with a 1:5 or 1:10 dilution of Asc.

†Immediately before Asc challenge.

‡Maximal response after Asc, usually occurring within 10 min after *Ascaris* administration.

major limitation of this model.¹² In the present study, responses to Asc were obtained in cynomolgus monkeys and these results were compared with responses in rhesus monkeys.

A high percentage of the cynomolgus monkeys tested demonstrated cutaneous reactivity to Asc, and

most of the monkeys with cutaneous reactivity reacted to Asc aerosols. However, as with rhesus monkeys,³ there were a few cynomolgus monkeys with cutaneous reactivity to Asc that lacked respiratory reactivity to Asc aerosols. The occurrence of animals with skin reactivity that did not react to Asc aerosols suggests that some other factor is necessary for bronchial reactivity. Furthermore, as has been reported in the rhesus monkey,³ no cynomolgus monkey in the present study with negative cutaneous reactivity to Asc reacted to repeated Asc aerosols.

The molecular weight of nematode allergens, including Asc, has been estimated to be 10,000 to 50,000.¹⁷ In order to test for a nonspecific reaction to a protein, several animals of both species were tested for skin and respiratory reactivity to OV (molecular weight $34,500 \pm 1,000$ ¹⁸). The weak cutaneous responses observed to 1.0% OV were probably due to a local inflammatory response to the introduction of a high concentration of a foreign substance under the skin, since skin responses were not observed with lower concentrations. When Asc-sensitive and Asc-nonsensitive rhesus and cynomolgus monkeys were exposed to an aerosol containing 2 mg/ml OV (a concentration approximately equivalent in terms of milligrams protein/milliliter to the highest concentration of Asc aerosolized in the experiments reported here), no respiratory responses were evoked. The above results agree with other reports^{2, 3} and support the idea that the responses observed in the present study to Asc

in responding monkeys of both species were due to the presence of a specific immediate-type hypersensitivity to Asc.

The changes in respiratory function observed after an Asc challenge in both rhesus and cynomolgus monkeys were similar to those reported by others in the rhesus monkey.^{3, 8-11, 19} Shortly after the Asc challenge, f increased, and $\dot{V}$, V_T , and C_{dyn} decreased. There was either an increase or very little change in R_L . The variability observed in R_L changes following Asc challenge in the cynomolgus monkeys in the present study has been noted by others in the rhesus monkey.^{8, 19, 20} In one study, a significant increase in R_L was observed after an Asc challenge in responding rhesus monkeys in only 18 of 32 experiments.¹⁹ In our studies, an increase in R_L of at least 15% from baseline values was noted after an Asc challenge in nine of the 13 cynomolgus monkeys and two of the four rhesus monkeys that reacted to Asc aerosols, and this increase was reproducible in repeated experiments. Since an increase in R_L is desirable in an animal model of asthma, the above results indicate that the Asc-sensitive cynomolgus monkey is an additional appropriate model in studies of allergic respiratory responses.

In all of the rhesus and some of the cynomolgus monkeys in which responses to repetitive challenges of Asc were obtained, the response to a subsequent challenge was lesser in magnitude than the response to the first challenge. The diminished response to a second challenge has also been noted by others,^{11, 21} especially when the concentration of the Asc aerosol employed was above the threshold concentration, i.e., the minimal concentration of Asc required to produce a respiratory response in responding animals. When the threshold concentration was employed in repetitive Asc challenges, the second response was generally greater than the first.²¹ In the present study, the second response to an Asc challenge was either greater than or less than the initial response when the same concentration of Asc was administered to different cynomolgus monkeys; this may reflect the difference in sensitivity of the various animals to Asc.

After an Asc challenge, an increase in BP was observed in those monkeys of both species that reacted to Asc aerosols, although changes in HR were variable. The cause for the increase in BP is uncertain. Mediators such as histamine, bradykinin, and PGE_2 are known to produce a fall in BP, and it is likely SRS-A would have similar effects.²² Therefore, other liberated substances, perhaps vasoconstrictive agents released into the systemic circulation, may have been responsible for the effects on BP observed following antigen challenge in both species.

In summary, the results of the experiments reported here indicated that the Asc-sensitive cynomolgus monkey responds in a similar fashion to Asc-sensitive rhesus monkeys and may be an additional useful model for the study of allergic respiratory responses. Since it appears that a sizeable number of cynomolgus monkeys are reactive to Asc aerosols (i.e., if our colony is indicative of the population of cynomolgus monkeys) the availability of sensitive animals for study may be greatly increased.

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