Latex-induced dermal and pulmonary hypersensitivity in rabbits

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Background: *Natural rubber latex (NRL) causes immediate, IgE-mast cell–mediated reactions in sensitized individuals, especially among health care workers and children with spina bifida. The immunopathogenesis of the reaction is obscure.*

Methods: *To study the immunologic mechanisms of NRL allergy, rabbits were sensitized with two nonammoniated and one ammoniated NRL antigens. Subcutaneous and intratracheal injections were used to immunize rabbits. Intradermal skin tests, IgG antibodies against NRL in serum and lung lavage fluid, and pulmonary histologic features were evaluated.*

Results: *One of nine sensitized rabbits died of anaphylaxis immediately after the third intratracheal injection with nonammoniated NRL. All sensitized rabbits, but not the controls, showed an immediate wheal and flare reaction in intradermal skin testing with NRL antigens. A positive skin reaction was detected 6 and 48 hours after the intradermal injections in four of five sensitized rabbits. A significant increase in NRL-specific IgG antibodies was detected in serum and lung lavage fluid of immunized rabbits. Lung histologic evaluation of NRL-sensitized rabbits showed a granulomatous interstitial and bronchial inflammation with a predominance of eosinophils and histiocytes in both intratracheally and subcutaneously sensitized rabbits. Vasculitis, hypertrophia, and contraction of pulmonary arteries could be detected in sensitized animals.*

Conclusion: *The results of the first NRL rabbit model study indicate that eosinophils and IgE antibodies play a major role in the immunopathogenesis of NRL-induced allergy and anaphylaxis. A wide range of inflammatory responses detected in rabbits injected by subcutaneous route without intratracheal exposure suggests that NRL exposure may pose a risk for a subsequent systemic reaction. (J Allergy Clin Immunol 1994;94:891-902.)*

Key words: *Natural rubber latex, eosinophils, skin tests, lung lavage, pulmonary histology*

The most common clinical manifestations of natural rubber latex (NRL) allergy are contact urticaria,[1] rhinoconjunctivitis,[2] and bronchial asthma.[3-5] Systemic anaphylaxis caused by NRL has affected patients who have undergone multiple operations, including children with spina bifida.[6] Other individuals at risk for NRL allergy are health care workers, especially those in operating rooms

Abbreviations used

LF:	Lung lavage fluid
Mal-ag:	Ammoniated latex from Malaysian rubber trees
N-Ind-ag:	nonammoniated latex from India
N-Mal-ag:	Nonammoniated latex from Malaysia
NRL:	Natural rubber latex
OD:	Optical density
PBS:	Phosphate-buffered saline

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and laboratories.[7-9] The increasing frequency of occupational NRL allergy among health care workers is related to the increasing use of protective latex gloves or to changes in the antigenic properties of the glove proteins during manufacture.

Although the clinical symptoms of NRL allergy have been recognized and widely studied, the immunopathogenesis of the syndrome is still unclear. According to previously reported findings, IgE mast cell–mediated reactions with specific IgE to NRL and resulting mediator release occur in sensitized patients on subsequent exposure to NRL antigens.[10] Skin test reactivity to allergens has been used in clinical practice to diagnose sensitized patients.[7] Skin testing, however, must be performed with proper antigens and with utmost care because of the risk of anaphylaxis.[11]

To understand the immunologic mechanisms in NRL allergy, we developed an animal model in rabbits. The rabbits were sensitized with antigens of ammoniated and nonammoniated Malaysian and Indian rubber tree *(Hevea brasiliensis)* sap, after which the animals developed NRL hypersensitivity as demonstrated by skin tests, production of specific NRL antibodies both in serum and lavage fluid (LF), and cosinophilic granulomas and interstitial inflammation in the pulmonary parenchyma.

METHODS

Animals

All procedures, such as the sensitization of experimental animals and processing of specimens, were carried out while nonlatex gloves were worn. Female New Zealand rabbits (n = 11), weighing 2.5 to 2.8 kg, were used in the experiments. Utmost care was taken to avoid undue pain to the rabbits, and the project was approved by the Institutional Animal Studies Committee.

Antigens

Three different NRL antigens were used in the immunization of the rabbits: (1) ammoniated NRL from Malaysian rubber trees (Mal-ag), (2) nonammoniated NRL from Malaysian rubber trees (N-Mal-ag), and (3) nonammoniated NRL from Indian rubber trees (N-Ind-ag). Characterization of the antigens was performed by estimating the protein content by the bicinchoninic acid method (Pierce Chemical Co., Rockford, Ill.), protein profile in polyacrylamide gels, and antigenicity by ELISA and crossed-immunoelectrophoresis.[12] The allergenicity of the NRL extracts was evaluated by skin prick tests in NRL-sensitive patients and normal control subjects.[11] The protein concentration of NRL antigens was as follows: Mal-ag, 1.5 mg/ml; N-Mal-ag, 5.6 mg/ml; and N-Ind-ag, 4.5 mg/ml. The antigens were stored at −70° C and thawed when needed.

Immunization of rabbits

Three groups of rabbits were immunized in this experiment (Table I). The first group (n = 3) was immunized subcutaneously with N-Ind-ag by introducing 1.4 mg of protein in 0.3 ml of phosphate-buffered saline (PBS) twice a week in two inguinal sites. The injections were repeated 10 times in a 2-month period. This was followed by intratracheal injection of 2.8 mg of the same protein in 0.5 ml of PBS through a 20-gauge needle at 10-day intervals for 6 weeks. The rabbits were anesthetized with intramuscular injection of ketamine (30 mg/kg) and xylazine (60 mg/kg) before the intratracheal injections with latex proteins.

The second group of rabbits (n = 3) was immunized by intratracheal injections with 2.8 mg of N-Mal-ag in 0.5 ml of PBS at 10-day intervals for 3 months, and the third group (n = 3) was immunized by subcutaneous injections with 0.45 mg of Mal-ag in 0.3 ml of PBS twice a week for 2 months. Control rabbits (n = 2) were anesthetized and injected intratracheally with 0.5 ml of saline solution.

Skin testing

The rabbits were skin tested intradermally with 100 μl of 1:10 and 1:100 dilutions of NRL extracts in sterile saline solution. Each animal was skin tested with the homologous antigen used for immunization. Cross-reactivity of N-Ind-ag and Mal-ag was tested in three rabbits immunized with N-Ind-ag and in two immunized with Mal-ag. A 1% histamine dihydrochloride solution was used as a positive control. Saline solution (100 μl) was used as a negative control. Skin reactions were observed at 30 minutes and at 6 and 48 hours after the intradermal injections. The diameter of the wheal and flare reaction was measured and recorded.

Antibody measurements

Serum samples were collected before immunization, after the 10 subcutaneous injections, and at the time of exsanguination. An LF sample from each rabbit was obtained at the termination of the experiment. ELISA was used to measure specific IgG antibodies to NRL both from the serum and LF samples of the rabbits, as previously described.[13, 14] Briefly, polystyrene microtiter plates coated with 1:1000 dilution of N-Mal-ag (in PBS) were treated with 1:500 dilutions of rabbit sera or undiluted LF. After 3 hours of incubation at 22° C and washing with PBS containing 0.05% Tween-20, heavy chain–specific biotinylated goat anti-rabbit antibodies against IgG (Sigma Immuno Chemicals, St. Louis, Mo.) were allowed to react with the rabbit sera for 1 hour. The wells were washed and treated with 1:10,000 dilution of peroxidase-labeled streptavidin (Sigma) in PBS containing 0.05% Tween-20. After further washing, enzyme activity was detected by using a peroxidase

TABLE I. Groups of rabbits and immunization schedule

Group	Antigen	Injection	*n*
1	N-Ind-ag	SC + IT	3
2	N-Mal-ag	IT	3
3	Mal-ag	SC	3
4	Saline	SC + IT	2

SC, Subcutaneous; *IT*, intratracheal.

substrate (o-phenylenediamine dihydrochloride, 0.8 mg/ml [Sigma]), and the optical density (OD) was read at 490 nm wavelength with an automated ELISA reader (Dynatech Laboratories, Inc., Alexandria, Va.).

Lung histologic features

Each rabbit was anesthetized intravenously with pentobarbital (20 mg/kg) and then exsanguinated through a carotid artery catheter. The chest was opened, and the right lung was excised for histologic and lavage studies. The middle lobe of the right lung was prepared for light microscopy, and the lower lobe for lung lavage. The fixation of the right middle lobe was performed by insufflating phosphate-buffered 4% formaldehyde–1% glutaraldehyde with a syringe into the lungs through a plastic cannula connected to the bronchus. After fixation through the airways, a piece of the lung tissue was immersed into 10% formalin. After paraffin embedding, 5 μm histologic sections were stained with hematoxylin-eosin. The morphologic features of lung tissue specimens were examined under a light microscope. The inflammatory changes in lung tissue were graded by using a scale from "−" to "+++," with one minus sign representing no change, one plus sign a mild change, two plus signs moderate change, and three plus signs a severe inflammatory change. The tissue specimens were evaluated independently by two investigators who did not know the exposure history of the examined animals.

Lung lavage

Lung lavage was performed through the bronchus of the lower lobe of the right lung. A 20-gauge needle surrounded by a plastic cannula was inserted into the bronchus, after which 5 ml of PBS was gently introduced into the lungs three times. Each time, the fluid was allowed to remain in the lungs for 30 seconds, after which the LF was aspirated with the same syringe. The LF was centrifuged at 1000 rpm for 10 minutes, and the supernatant was stored at −70° C for measurements of NRL antibodies. The sedimented cells were suspended in sterile PBS and prepared in a cytocentrifuge (Shandon Cytospin II; Shandon Scientific Ltd., Gb-Runcorn, Cheshire, U.K.) fixed in methanol for 10 minutes, and stained with a Diff-Quik (Baxter Healthcare Corp., Deerfield, Ill.) staining set. The cell populations were examined microscopically, and the different types were enumerated.

Statistical analysis

The means of the results of antibody measurements and lung lavage cell counts in the immunized rabbits and controls were compared by an unpaired Student's *t* test. A probability value of 0.05 or less was used for statistical significance.

RESULTS

All rabbits sensitized with NRL antigens developed hypersensitivity, which could be detected by skin tests, demonstration of NRL-specific IgG antibodies in serum and LF, and the characteristic lung histologic features; whereas the control rabbits injected subcutaneously or intratracheally with saline solution remained healthy (Tables II and III).

One sensitized rabbit (no. 7 in Table II) treated with three intratracheal injections of N-Mal-ag showed NRL-specific IgG antibodies in serum and died of anaphylaxis after the third injection. This rabbit had severe tachypnea and bradycardia 3 to 5 minutes after the injection and became apneic and asystolic 10 minutes after the injection. A significant decrease in specific IgG to NRL was detected in the serum of this rabbit 30 minutes after the intratracheal injection of NRL antigen compared with the serum IgG levels before the last injection (Fig. 1). The LF specimen of this rabbit showed the lowest value of NRL antibodies (OD = 0.892) of all sensitized rabbits in this experiment. Lung histologic examination of the anaphylactic rabbit revealed alveolar exudate, interstitial edema, and hemorrhage with eosinophilic and macrophage infiltration. To evaluate this phenomenon, another two rabbits were given three intratracheal injections of N-Mal-ag. Serum samples were collected before the immunization and just before and after the third injection. There was a significant increase in IgG antibodies to NRL in the sera of these two rabbits, but they

TABLE II. Histologic findings and antibody measurements in NRL-immunized rabbits and controls

Finding	Ind-ag			Mal-ag			NMal-ag			Control	
	1	2 (SC + IT)	3	4	5 (SC)	6	7	8 (IT)	9	10	11
Histologic*											
Edema											
Interstitial	+	+	+ +	−	+	−	+	+ +	+ +	−	−
Alveolar	+ +	+	+ +	−	−	−	+	+ +	+ +	−	−
Inflammation											
Interstitial	+ + +	+ +	+ + +	+	+	+	+ +	+ +	+ +	−	−
Alveolar	+ + +	+ +	+ + +	−	−	−	+	+ +	+ +	−	−
Bronchiolar	+ +	+ +	+	−	−	−	+	+ + +	+ +	−	−
Granulomas											
Parenchymal	+ +	+ +	+ +	+	+	+	−	+	+	−	−
Bronchiolar	+ + +	+ +	+ +	−	−	−	−	+	+	−	−
Fibrosis											
Interstitial	−	−	−	−	−	−	−	−	−	−	−
Alveolar	−	−	−	−	−	−	−	−	−	−	−
Vascular changes											
Thick media	+	+	+ +	−	+	−	+ +	+	+	−	−
Vasculitis	+	−	+	+	+	−	−	+	+	−	−
Antibodies to NRL†											
Serum antibodies (OD value)	1.379	1.504	1.392	1.419	1.389	1.437	0.250	1.307	1.457	0.537	0.286
Lavage antibodies (OD value)	1.150	1.140	1.161	1.189	1.192	1.096	0.892	1.222	1.185	0.584	0.437

SC, Subcutaneous; *IT,* intratracheal.
*Histologic changes in lungs of rabbits sensitized with latex. Scale from − to + + + (− = not found, + = mild, + + = moderate and + + + = severe change).
†Serum and lung lavage antibodies were measured by ELISA, and results are expressed as OD^{490}.

TABLE III. Percentage of different cells in lavage fluid in NRL-sensitized and control rabbits

Lavage cells	Group 1 (SC + IT)		Group 2 (SC)		Group 3 (IT)		Control	
	%	SEM	%	SEM	%	SEM	%	SEM
Macrophages	73	1.5*	84	3.4*	86	0.8*	96	0.3
Eosinophils	12	0.5*	8.1	3.3*	4.4	0.6*	0.5	0.5
Lymphocytes	8.5	0.7*	5.6	0.8	3.8	0.5	0.5	0.5
Neutrophils	6.2	0.5	2.4	0.8	5.4	0.7	3.7	0.2

SC, Subcutaneous injection with antigen; *IT,* intratracheal injection with antigen; *SEM,* standard error of the mean.
*$p < 0.05$ compared with control values.

did not show any decrease in the antibodies 30 minutes after the third injection. The two rabbits did not show any signs of anaphylaxis after the antigen injections.

Skin testing

All rabbits sensitized with NRL antigens showed an immediate skin reaction when tested with the NRL antigen. Cross-reactivity of N-Ind-ag and Mal-ag was tested in five rabbits. In three rabbits immunized with N-Ind-ag, a positive skin reaction was detected at 30 minutes and 6 and 48 hours after the intradermal injection with N-Ind-ag. In two of three rabbits, Mal-ag caused a positive reaction at 30 minutes and 6 and 48 hours after the injection, whereas in one rabbit, only the 30-minute and 6-hour reactions were positive. In two rabbits immunized with Mal-ag, a positive skin reaction was detected 30 minutes and 6 hours after injection of Mal-ag, and one showed a positive reaction 48

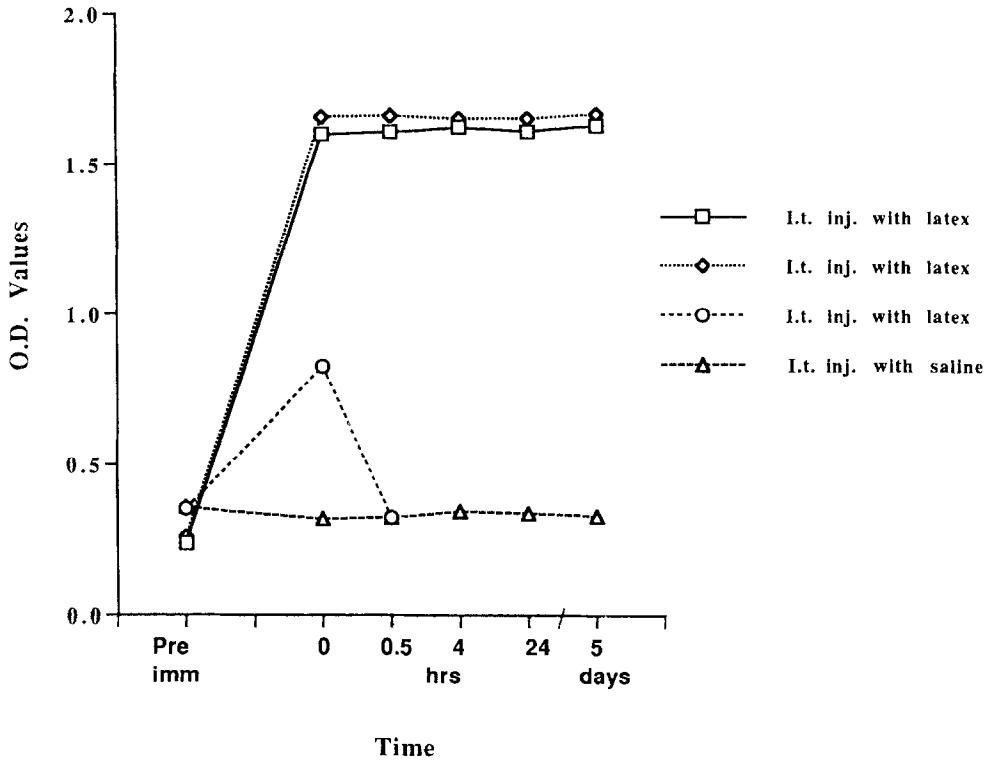

FIG. 1. NRL-specific IgG in rabbit sera. Three rabbits each were injected with latex or saline solution. One rabbit had anaphylaxis and a decrease in NRL-specific IgG level in serum after the third intratracheal injection with NRL.

hours after the test. N-Ind-ag produced a positive reaction in both rabbits at 30 minutes and 6 and 48 hours after injection, indicating superior allergenicity compared with the other two antigens.

Serum and LF antibodies

The results of specific antibody measurements in response to N-Mal-ag in rabbit serum and LF are shown in Fig. 2. A significant increase in NRL-specific IgG was detected in sera of sensitized rabbits compared with preimmunized serum samples and the sera of control animals ($p < 0.001$). Low levels of antibodies to NRL were detectable in preimmunized animals and controls.

High titers of IgG antibodies to NRL were detected in lavage fluid of sensitized rabbits. There was a threefold increase in the mean OD values of antibodies in sensitized rabbits compared with controls ($p < 0.001$).

Lung lavage cells

The percentages of different cells detected in LF are shown in Table III. Normal control rabbits showed predominance of macrophages (96%, SEM = 0.3), whereas other cell types were scanty. In group 1 in which the animals received both subcutaneous and intratracheal injections of antigens, the percentage of macrophages showed a decrease (73%, SEM = 1.5), whereas other cell types, especially eosinophils, showed a significant increase. In all sensitized animals the percentage of eosinophils and lymphocytes was higher than in control rabbits, and in group 1 the difference was statistically significant ($p < 0.05$).

Histologic findings

The histologic changes in lung tissue of rabbits are shown in Table II. All sensitized animals showed at least mild inflammatory changes in the lung tissue, irrespective of the antigen or immunization route that had been used. The lungs of normal control rabbits did not show any pathologic changes (Fig. 3).

In sensitized rabbits interstitial, alveolar, and bronchiolar inflammation varied from mild to severe. In most of the rabbits, both alveolar and interstitial edema could be detected. The most common finding in sensitized rabbits was peribronchiolar and perivascular inflammation with

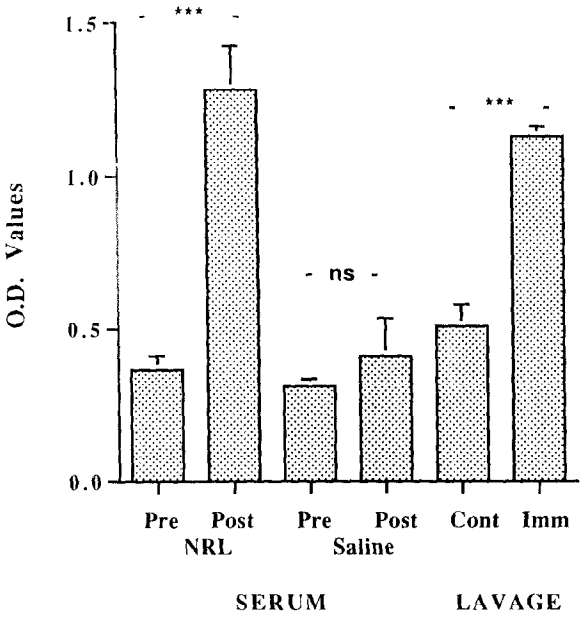

FIG. 2. NRL-specific IgG in serum and LF before and after the immunization in NRL-exposed rabbits ($n = 9$) and saline-injected controls ($n = 2$).

eosinophils, histiocytes, lymphocytes, and plasma cells. Bronchiolar and bronchial inflammation consisting of mucous fluid in the lumen of the airways, and eosinophils in the bronchiolar walls was occasionally present in the airways of sensitized rabbits. In sensitized animals destruction and detachment of bronchiolar epithelium was often detected, and in some animals peribronchiolar inflammation with eosinophils and histiocytes could be found in the basal membrane of the bronchiolar wall (Fig. 4). Inflammatory changes in lung parenchyma in general were mostly focal.

The predominance of eosinophilic granulocytes was demonstrated in the lungs of the sensitized rabbits. Numerous large cells with single nuclei and double or multinucleated giant cells could be detected in the alveolar lumina and occasionally in the interstitium (Fig. 5). The cytoplasm of these cells primarily contains eosinophilic granules. Granulomas (usually consisting of eosinophils, lymphocytes, and histiocytes, and occasionally giant cells) were observed in the lung tissue (Fig. 6). Granulomas were present in the parenchyma of the lungs of both subcutaneously and intratracheally injected animals, but not in the bronchiolar wall of rabbits sensitized by subcutaneous injections.

The rabbits immunized with intratracheal injections had bronchiolitis, which was often accompanied by granulomas in the bronchiolar wall, whereas the subcutaneously injected animals showed peribronchiolar and perivascular pulmonary inflammatory changes (Fig. 7).

No fibrosis or necrosis of the granulomas was present in the lungs of exposed animals. Thickening of the media of the pulmonary arteries was detected in all rabbits exposed to N-Ind-ag and N-Mal-ag and in one of three animals sensitized subcutaneously with Mal-ag. In two animals lung biopsy specimens were taken before and after the lungs were excised. In the specimens that were obtained after the NRL antigen was injected into the pulmonary veins or trachea of the excised lung of the sensitized rabbits, a contraction of pulmonary arteries could be detected compared with the noncontracted vessels in the biopsy specimens in the same rabbits before the antigen injections (Figs. 8 and 9). Vasculitis with eosinophilic and lymphocytic infiltration in the walls of small vessels could be detected in animals immunized with NRL antigens.

DISCUSSION

Although a murine model of latex allergy has been reported, to our knowledge this is the first experimental study concerning NRL allergy in a rabbit model.[14] The model was designed to characterize the basic inflammatory changes in sensitized animals receiving three NRL antigens through two different routes. All rabbits immunized either with subcutaneous or intratracheal injections of NRL developed hypersensitivity. In this experiment differences in the immunologic responses caused by three antigens could be found in the histologic features of sensitized rabbits. However, rabbits sensitized with ammoniated latex showed milder inflammatory changes in the lungs compared with the animals sensitized with nonammoniated latex. It is noteworthy that both nonammoniated antigens had higher protein and perhaps higher antigen concentrations, which may partly explain the differences in the lung damage observed. An alternate explanation may be that the ammoniated latex was injected subcutaneously in the rabbits, which may not cause as severe inflammation as the combined subcutaneous and intratracheal route. The findings suggest, however, that it may be worthwhile to carry out systematic studies to determine whether the inflammatory responses may vary because of the treatment of raw NRL with ammonia.

We selected three different NRL antigens of rubber tree sap extracts from two countries, India and Malaysia, for the sensitization procedures in

FIG. 3. A normal lung tissue sample from a rabbit injected intratracheally with saline solution. (Hematoxylin-eosin stain; ×50.)

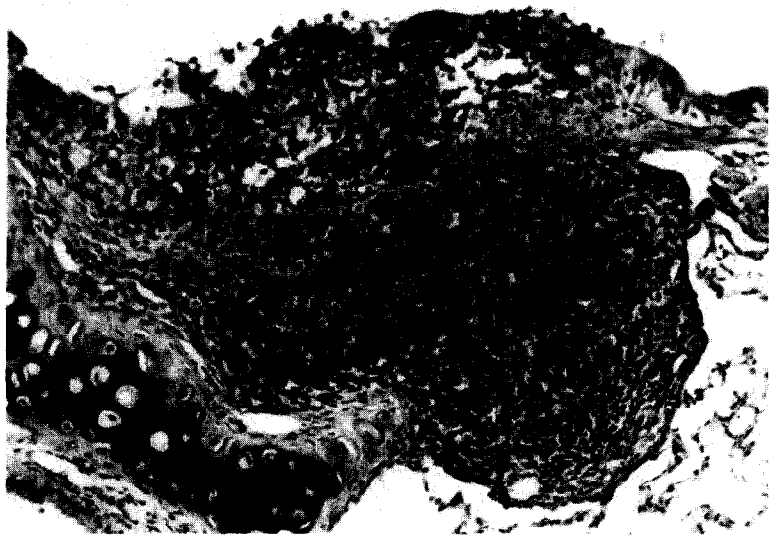

FIG. 4. Peribronchial aggregation of inflammatory cells consisting of eosinophils, histiocytes, lymphocytes, and plasma cells in an NRL-immunized rabbit. The inflammatory process is protruding into the basal membrane of the bronchial wall. Note the detachment of the bronchial epithelium in the area of inflammation. (Hematoxylin-eosin stain, ×160.)

order to study possible differences of these antigens in their capability of causing inflammatory responses. According to earlier studies, the response to latex may vary in sensitized individuals, depending on the antigen source.[7] Results of unpublished studies in our laboratory have shown that ammoniated latex is immunologically less potent than nonammoniated latex, suggesting different antigenicity in these two materials.

Rabbits were used because an attempt was made to develop an animal model in which skin test reactivity, lung inflammatory responses, and physiologic functions could be examined in the same animals. The advantage of the rabbit model is that the size of the animal is optimal for physiologic studies, as well as for lung lavage and histologic investigations. This potential of the rabbit model for physiologic studies was the primary

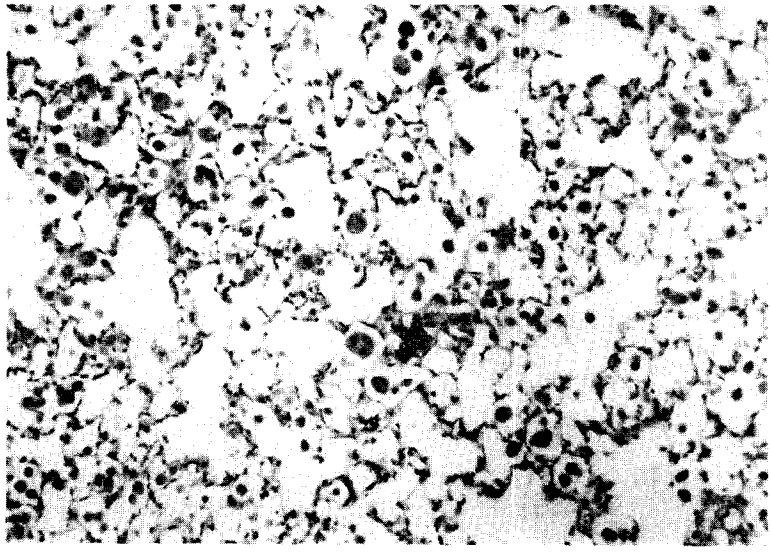

FIG. 5. Intra-alveolar inflammation with large giant cells containing eosinophilic granular material. Interstitium and alveolar walls show a mild inflammation in rabbit lung immunized with intratracheal injections of NRL. (Hematoxylin-eosin stain; ×160.)

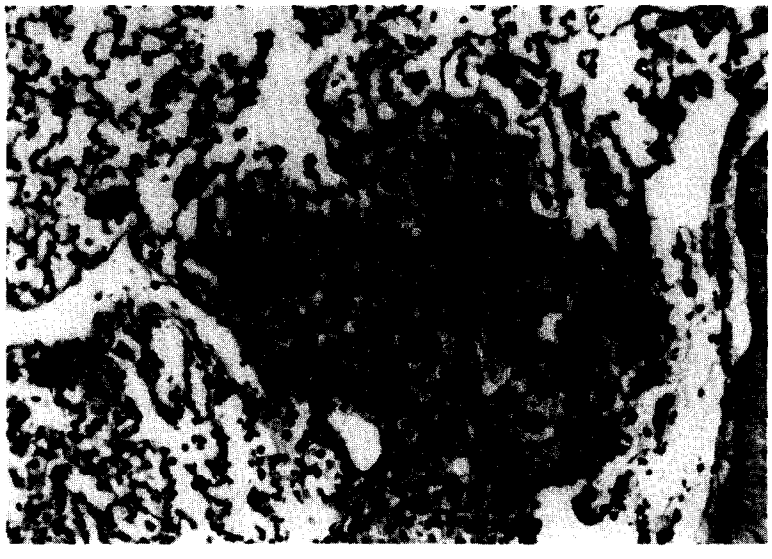

FIG. 6. Perivascular granuloma with numerous eosinophils, histiocytes, and lymphocytes in a rabbit immunized with subcutaneous and intratracheal injections of NRL. Eosinophilic infiltration is visible around the arterial wall. (Hematoxylin-eosin stain; ×160.)

reason for pursuing these studies in this species. The disadvantage of the rabbit model is the lack of immunologic reagents available compared with murine models. According to recent literature, the rabbit model of anaphylaxis has been successfully used to study the physiologic changes in IgE-dependent anaphylactic reactions.[15]

Two different routes of antigen exposure were selected in this experiment to determine whether either route was effective in producing hypersensitivity to NRL antigens in rabbits. Although the majority of NRL allergy symptoms in human beings occur as contact urticaria, respiratory symptoms associated with the use of latex gloves have been reported in several studies.[4, 7, 9] It was interesting to find that subcutaneous injections alone were able to cause inflammatory changes in the lungs of sensitized animals. This raises questions

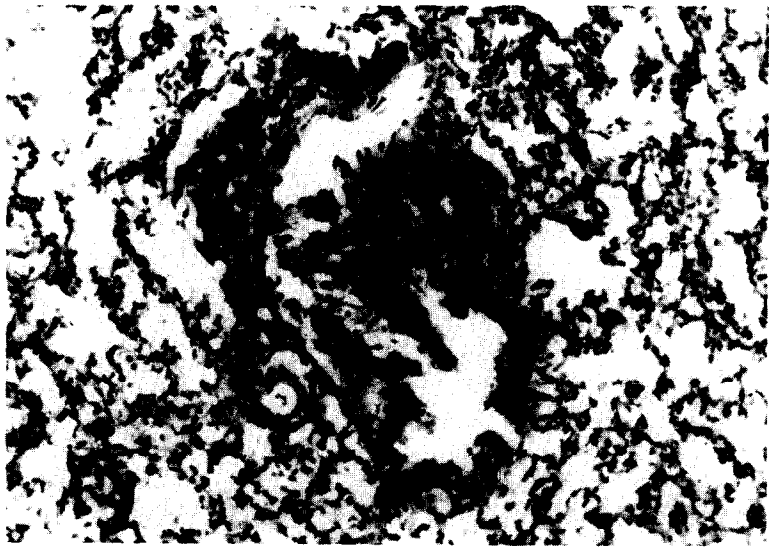

FIG. 7. Granuloma with eosinophils, lymphocytes, and histiocytes between a respiratory bronchiole and a small artery in a rabbit that had intratracheal injections of NRL. Note the perivascular eosinophils around the artery. (Hematoxylin-eosin stain; ×160.)

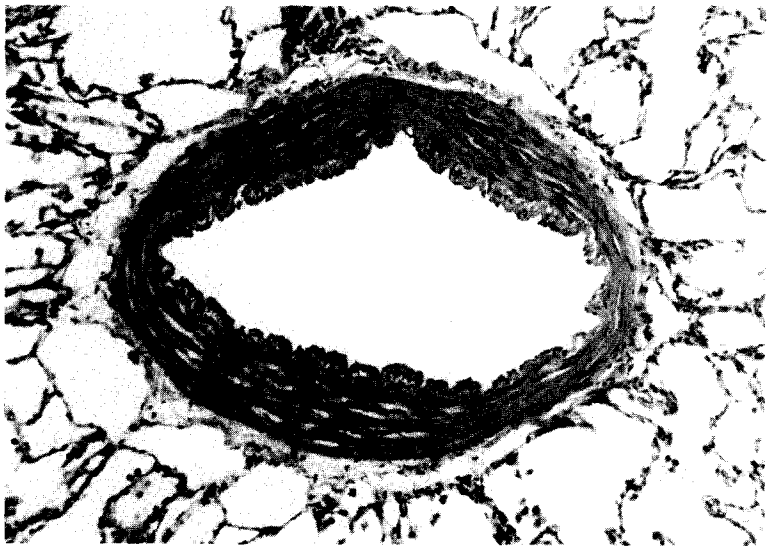

FIG. 8. Normal histologic features of a pulmonary artery of an NRL-sensitized rabbit before NRL exposure. (Hematoxylin-eosin stain; ×160.)

about the safety of skin tests with NRL antigens in human beings because repeated skin tests with NRL might also result in systemic reactions with anaphylaxis.

Skin testing of exposed patients has been widely used in the diagnosis of NRL allergy.[7] Dependable and reliable skin tests can only be performed by using relevant and well-characterized antigens. There has been a discrepancy between the findings in skin tests and antibody measurements, which may be explained in part by the use of uncharacterized antigens.[8] Furthermore, there is a risk of anaphylaxis during skin testing when potent test reagents are used in sensitized subjects.[6, 11] An animal model such as this may be useful for identifying relevant antigen when purified NRL test reagents become available. It may also provide some insight into the issues of safety of skin test reagents and epitope specificity.

High titers of IgG antibodies against NRL were

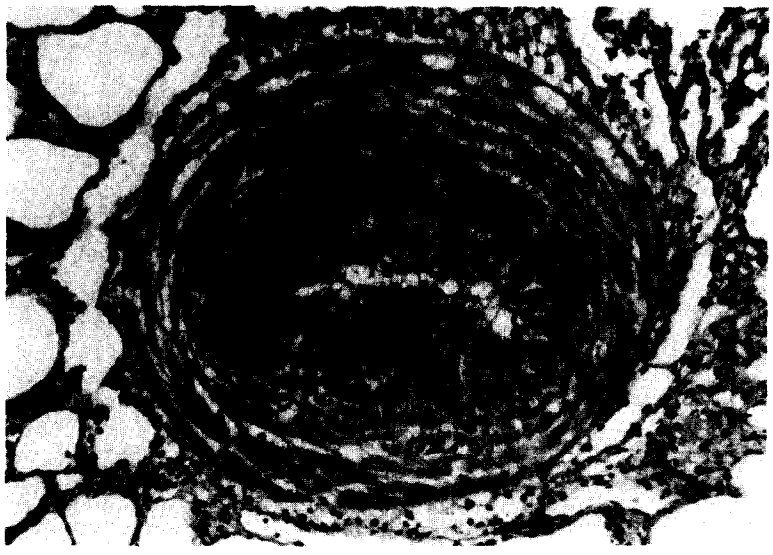

FIG. 9. A contracted pulmonary artery in the same rabbit as in Fig. 8. Severe contraction of the artery, thickening of the media of the vessel wall, and numerous eosinophils around the vessel can be detected. (Hematoxylin-eosin stain; ×160.)

detected in serum and LF of sensitized animals. The presence of IgE antibodies was obvious because an immediate skin reaction was detectable in all sensitized animals with NRL antigens. Eosinophilia was also evident in lung tissue and LF after the final exposure to the antigens. The presence of eosinophils, neutrophils, and lymphocytes in lung tissue of sensitized rabbits suggests that in this model of NRL allergy, there is an immediate IgE and eosinophil response, which might be accompanied by lymphocyte and neutrophil infiltration after the exposure.

In one intratracheally injected animal (no. 7 in Table II) without any previous subcutaneous injections of NRL antigen, respiratory failure developed after the third intratracheal injection. The symptoms induced and the histologic findings provided evidence that in this sensitized animal anaphylaxis developed after the NRL injections. A significant decrease in the NRL-specific antibodies in both serum and LF could be detected in this rabbit only and not in two similarly injected controls. The antibody levels of these animals did not decrease after the third antigen injection. Thus our findings suggest that specific IgG antibodies may play a role in protecting immunized individuals from anaphylaxis after NRL exposure.

Lung tissue of sensitized rabbits showed a unique granuloma formation: parenchymal and bronchiolar granulomas consisted of numerous eosinophilic granulocytes and histiocytes but only a few lymphocytes. This differs significantly from the granulomas found in hypersensitivity pneumonitis in which the granulomas are mainly formed by lymphocytes, macrophages, plasma cells, and giant cells.[16, 17] Eosinophilic granulocytes are not typical in the granulomatous inflammatory lung process.[16] Large histiocytes filled with eosinophilic material have been demonstrated in mice challenged with *Aspergillus fumigatus* and latex antigens.[14, 17] Obviously, these cells either contain or have digested material liberated from eosinophilic granulocytes and may play a significant role in the inflammatory process caused by latex. In NRL-induced eosinophilic lung inflammation, bronchiolitis and bronchiolar epithelial damage could be detected in the sensitized rabbits. This suggests a major role for eosinophils in bronchiolar inflammation, which may lead to the typical epithelial destruction found in early stages of bronchial asthma and hypersensitivity pneumonitis.[18, 19] Further investigations with electron microscopy are needed, however, to describe the fine structure of latex-induced epithelial changes in the bronchi and bronchioli.

Clinical symptoms of NRL allergy such as contact urticaria, rhinitis, asthma, and anaphylaxis suggest that IgE-mediated immune responses with eosinophilia are involved in the inflammatory process.[10] The data from this study indicate an

IgE-mediated reaction, as demonstrated by an immediate positive skin test response to NRL antigens. Furthermore, the presence of eosinophilia in the lung tissue was confirmed in earlier studies of murine NRL allergy.[14] Interestingly, NRL was also able to cause Arthus and delayed-type skin reactions, suggesting a role for lymphocytes and antibodies in the immunologic response. So far, this has not been demonstrated in human NRL allergy because most of the skin tests in NRL allergy have been skin prick tests and no skin biopsy specimens have been available for histologic studies. In the lung tissue the number of lymphocytes was only slightly increased. Eosinophilic granulocytes appear to play a major role in the inflammatory process of rabbit NRL allergy. The high number of eosinophils detected around those pulmonary arteries with contraction may suggest that eosinophils locally orchestrate the vasoconstriction that leads to an increase in the pulmonary arterial pressure in rabbit anaphylaxis.

Immediate hypersensitivity in rabbits is characterized by pulmonary manifestations, particularly pulmonary vasoconstriction. From the standpoint of the lungs, repeated subcutaneous injections may be equivalent to repeated intravenous injections in that the antigens reaching the lungs through the blood stream may produce the release of mediators within the lungs, which in turn may lead to chronic pulmonary vascular changes such as arterial wall thickening and hypertrophy.

Clinical manifestations such as immediate reaction in the anaphylactic rabbit, immediate skin reactions, and eosinophilic infiltration in lung tissue suggest that type I reaction with IgE-mediated inflammation plays a major role in the immunopathogenesis of NRL-induced hypersensitivity. However, the presence of NRL-specific IgG antibodies in both serum and lavage fluid in exposed animals, delayed skin reactions caused by NRL, and granulomatous inflammation with a prominent eosinophilic and histiocytic infiltration in the lung tissue suggest that both cell-mediated and immune complex–mediated immunity may also be involved in NRL allergy. Thus the immune responses noted in this model constitute a mixture of immediate, late, and delayed hypersensitivity reactions to antigens. Because of the lack of availability of information on histologic changes in NRL-induced hypersensitivity in human beings, it may not be possible to ascertain the validity of the present rabbit model of latex allergy and make meaningful comparisons. Our model showed resemblances to and minor differences from other models and human hypersensitivity pneumonitis. However, it can be concluded that the immune response noted in this model is complex and comparable to the responses noted in the murine model.[14]

We conclude that the findings in this model of NRL allergy in rabbits clearly demonstrate the major role of eosinophils and IgE in association with lymphocytes and neutrophils in the immune response. The unique granulomatous response with eosinophilic granulomas in lungs caused by NRL, even in the rabbits that were only receiving subcutaneous injections of NRL antigen, suggest that further studies are needed to confirm the safety of intradermal skin testing.

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Passive transfer of cutaneous mosquito-bite hypersensitivity by IgE anti-saliva antibodies

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Background: *Mosquito bites frequently cause cutaneous wheal and flare reactions, and recent immunoblotting studies have shown specific anti-saliva IgE antibodies in many persons who have such reactions.*
Objective: *The study was designed to show that human serum containing mosquito saliva–specific IgE antibodies can produce histamine release in vitro and whealing in vivo.*
Methods: *Two mosquito bite–tolerant subjects had bite challenges and Prausnitz-Küstner tests with heated and unheated serum from one patient with* Aedes *mosquito allergy. Immunoblotting and basophil histamine release tests were performed with the patient's and subjects' sera.*
Results: *Both mosquito bite–tolerant subjects had positive Prausnitz-Küstner reactions, which indicated a successful transfer of cutaneous mosquito hypersensitivity. The ordinary and passive basophil histamine release tests also produced positive results with* Aedes communis *antigens.*
Conclusion: *The results of the Prausnitz-Küstner test, immunoblotting, and basophil histamine release tests are consistent with the hypothesis that mosquito bite whealing is mediated by specific anti-saliva IgE antibodies. (J ALLERGY CLIN IMMUNOL 1994;94:902-6.)*

Key words: *Mosquito allergy, IgE and IgG_4 antibodies, Prausnitz-Küstner test; basophil histamine release test*

Mosquito bites cause cutaneous wheal and flare reactions and delayed bite papules in most persons, but in contrast to bee and wasp stings, anaphylactic reactions are extremely rare.[1, 2] Evidence that whealing and the rare immediate systemic symptoms caused by mosquito bites are mediated by IgE antibodies, as they are in Hymenoptera and other insect allergies, is increasing. Studies in animals and human beings have demonstrated successful passive transfer of immediate mosquito hypersensitivity.[3-5] A few studies have detected IgE antibodies to mosquitoes in

Abbreviations used
BHRT: Basophil histamine release test
P-K: Prausnitz-Küstner
WBE: Whole-body extract

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