

THE JOURNAL OF Allergy AND Clinical Immunology

January 1997 • Volume 99 • Number 1

Failure of aged rats to accumulate eosinophils in allergic inflammation of the airway

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Abstract

TOP

To investigate the effect of aging on the allergic airway response, we examined the bronchoconstrictive responses and cellular inflammatory changes in a rat model of bronchial asthma by evaluating young and old animals. Two different age groups of Brown-Norway rats, actively sensitized by injection of ovalbumin into the foot pads, were used: 7 to 8 weeks old (young group) and 100 to 120 weeks old (aged group). Both the aged and young rats produced an ovalbumin-specific IgE antibody and exhibited an immediate asthmatic response after exposure to

ovalbumin, but the degree of specific IgE antibody was significantly higher in young rats. The young group showed a marked increase in the number of eosinophils and neutrophils in bronchoalveolar lavage fluid 2 days after exposure to ovalbumin, whereas no eosinophilia was seen in the aged group. To evaluate the mechanism of the decreased accumulation of eosinophils in aged rats, cells from popliteal lymph nodes from ovalbumin-sensitized rats were incubated with ovalbumin for 48 hours. Although eosinophil chemotactic activity, determined by a modified Boyden chamber method, was present in the supernatant of cultured lymph node cells from young rats, it was absent from those of aged rats. In vivo administration of anti-IL-5 monoclonal antibody revealed that one of the factors of eosinophil chemotactic activity was IL-5. Lymph node cells from aged rats tended to produce greater amounts of interferon- γ than did those from young animals. Findings indicate that aged rats have a defect in eosinophil accumulation in sites exposed to antigen, probably because of an age-dependent alteration in T cells. (J Allergy Clin Immunol 1997;99:38-47.)

BALF	Bronchoalveolar lavage fluid
ECA	Eosinophil chemotactic activity
IFN-γ	Interferon- γ
LN	Lymph node
PBS	Phosphate-buffered saline
RL	Lung resistance

(Click on a term to search this journal for other articles containing that term.)

Key Words: [Aging](#), [bronchial asthma](#), [eosinophil](#), [lymphocyte](#), [rat](#)

Asthma is characterized by reversible airflow obstruction, bronchial hyperresponsiveness, and a narrowing of the airway in response to various exogenous and endogenous stimuli. Inflammation of the airway is characterized by infiltration of the bronchial mucosa with large numbers of inflammatory cells such as lymphocytes, eosinophils, macrophages, and mast cells and by the presence of elevated concentrations of eosinophil-derived proteins. The latter play a central role in the etiology of asthma.¹⁻⁴

Allergic disorders such as bronchial asthma, allergic rhinitis, and atopic dermatitis (eczema) usually begin in childhood or early in adult life. Detection of allergies becomes less frequent with age, and symptoms tend to decrease.⁵ Aging may therefore represent an interesting in vivo model for studying the waning of allergic response.

Information about age-dependent immunologic phenomena is available. Serum IgE level and antigen-specific IgE production both decline with age,⁶⁻⁸ which may help to explain the age-dependent decline in allergic disease. Numerous reports have dealt with age-related changes in the cytokine profile of T cells stimulated with nonspecific stimulants in a variety of species.⁹⁻¹² How aging modifies allergic inflammation after antigenic stimulation is not well understood.

The age-dependent decrease in allergic reactions led us to establish an experimental model in aged animals for investigating the inhibitory mechanism in the development of allergic inflammation.

We first compared the bronchoconstrictive reaction in young and aged rat models of bronchial asthma. We also investigated the influx of inflammatory cells into the airway after antigenic challenge and measured the eosinophil chemotactic activity (ECA) that was generated by lymph node (LN) cells. Our results indicate that aged rats are defective with respect to eosinophil accumulation in antigen-exposed sites, which is probably due to age-dependent alteration in T-cell functions.

Methods

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Animals

Two different age groups of inbred male Brown-Norway rats were obtained from Charles River (Tokyo, Japan): 7 to 8 weeks old (young group) and 100 to 120 weeks old (aged group). Rats were kept in the animal research facility of Hamamatsu University School of Medicine in an air-filtered, pathogen-free environment during these experiments.

Sensitization and challenge

Both groups of rats were actively sensitized by a subcutaneous injection of ovalbumin, 0.25 mg/kg (Sigma Chemical Co., St. Louis, Mo.) and $\text{Al}(\text{OH})_3$, 20 mg/kg (a generous gift from Dr. H. Nagai, Gifu Pharmaceutical University, Japan), in borate-buffered saline solution into two foot pads of each rat. At the same time, 0.5 ml of *Bordetella pertussis* vaccine (Wako Pure Chemical Industries Ltd., Osaka, Japan) containing 1×10^{10} heat-killed bacilli in phosphate-buffered saline (PBS), pH 7.4, was administered intraperitoneally as an adjuvant. After 7 days, ovalbumin in PBS, 0.125 mg/kg, was injected subcutaneously. Fourteen days after the initial injection, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium, 30 to 40 mg/kg. A tracheal catheter (14G Therflow; Telmo, Tokyo, Japan) was placed in the trachea by oral intubation, and the rats were challenged with a 5% ovalbumin aerosol, which was generated by an M2 nebulizer (Shin-ei Industries, Nagoya, Japan) run on compressed air.

Measurement of lung resistance

Rats were anesthetized and intubated, as described above, and additional pentobarbital was administered as required to maintain anesthesia. They were placed in a supine position, and a heating pad was used to maintain a constant body temperature. To measure airflow during spontaneous breathing, a pneumo-tachograph (TV-241T; Nihonkhoden, Tokyo, Japan) coupled to a differential pressure transducer (TP-602T, Nihonkhoden) was connected to the end of the tracheal catheter. Volume was determined by electrical integration of the flow signal with a respiratory integrator (AQ-601; AR-601G, Nihonkhoden). Transpulmonary pressure was measured at the same time with a water-filled catheter (Multi Purpose Tube; ATOM, Tokyo, Japan) placed in the lower portion of the esophagus and connected to a pressure transducer (DX360DT; AP601G, Nihonkhoden). The esophageal catheter was advanced into the esophagus until the cardiac artifact

on the transpulmonary pressure tracing was minimized. Lung resistance (RL) was calculated by the isovolume method.¹³ Four consecutive breaths were measured, and a mean value was obtained.

Rats were challenged initially with a saline solution aerosol for 10 minutes; the subsequent RL value was used as a control. Each animal was then challenged with 5% ovalbumin, and RL was measured 1, 3, 5, 10, 20, and 30 minutes after exposure and every hour thereafter for 10 hours. RL was expressed as a percentage of the control RL value.

Antibody determination

Levels of serum IgE specific to ovalbumin were determined by ELISA, as described by Wasserman et al.¹⁴ All assays were performed on 96-well microtiter plates (Nunc A/S, Roskilde, Denmark). Plates were coated overnight with mouse monoclonal antibody to rat IgE, 2 µg/ml, (Zymed Laboratories Inc., San Francisco, Calif.) diluted in carbonate/bicarbonate buffer. Plates were blocked with PBS containing 0.5% casein (PBS-casein) for 1 hour at 37° C. Test sera were diluted 20 times in PBS-casein. Wells containing the diluted sera were incubated at 4° C overnight. After the incubation, biotin-labeled ovalbumin (5 µg/ml) in PBS-casein was added to the wells, followed by another overnight incubation. Subsequently, alkaline phosphatase–conjugated streptavidin (Zymed) in PBS-casein (1:1000) was added and incubated for 1 hour at 37° C. Between steps, the wells were washed four times with 0.05% Tween-20 (Sigma) in PBS. Plates were developed at room temperature after the addition of *p*-nitrophenyl phosphate in diethanolamine buffer (1 mg/ml) and read with a microplate reader (Toyo, Zama, Japan) at 405 nm.

Rat ovalbumin-specific IgE was not commercially available, so the results of specific immunoglobulin assays were expressed in arbitrary units of absorbance. It was confirmed empirically that the absorbance units of doubled concentrations of diluted test samples represented doubled values.

Bronchoalveolar lavage and cell counting

Blood samples were obtained for antibody determination 2 days after ovalbumin challenge. The anesthetized animals were then killed by exsanguination. The trachea was clamped, and the thoracic cage was opened through the diaphragm. The lungs were removed intact and lavaged with 5 × 2 ml of Hanks' balanced salt solution (Sigma) through a polyethylene tube introduced into the trachea. Lavage fluids were centrifuged at 200 *g* for 10 minutes, and the cell pellets were resuspended in 1 ml of Hanks' balanced salt solution. Total cell counts were carried out with a hemocytometer. Differential cell counts were performed by use of cytopspin preparations subjected to May-Grünwald-Giemsa staining. In each sample 300 cells were identified by standard structure as alveolar macrophages, eosinophils, lymphocytes, neutrophils, or other cells.

Histologic examination

Lungs were fixed with 10% formaldehyde and stained with May-Grünwald-Giemsa. They were also fixed with 4% lead acetate (Katayama Chemical, Osaka, Japan) and stained with toluidine blue to detect mast cells. To evaluate the distribution of mast cells in the airway, we counted the number of mast cells in the epithelial layer (for epithelial mast cells) or in the area below it (for subepithelial mast cells). Results were expressed as follows: (minus sign), none; (plus sign), 1 to 3 cells; (two

plus signs), more than 3 cells in 25 μm of epithelial layer or beneath the epithelial area.

Culture of LN cells

Fourteen days after initial sensitization, rats were killed by ether anesthesia, and the draining popliteal LNs were excised aseptically. Cell suspensions of LN in N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid buffered RPMI-1640 containing penicillin, 100 U/ml, streptomycin, 100 $\mu\text{g}/\text{ml}$, sodium bicarbonate, 2 gm/L, 2 mmol/L L-glutamine, and 5% heat-treated fetal bovine serum (Gibco BRL) were prepared by mechanical disaggregation through a steel mesh. Cells were washed twice by centrifugation at 100 g for 10 minutes in Hanks' balanced salt solution, and resuspended at $1 \times 10^6/\text{ml}$ in RPMI-1640 medium with 5×10^{-5} mol/L 2-mercaptoethanol (Gibco). LN cells were incubated with ovalbumin at a final concentration of 1 mg/ml for 48 hours. Trypan blue exclusion revealed that cell viability exceeded 80% after the incubation. The culture media were centrifuged at 100 g for 15 minutes, and the supernatants thus obtained were stored at -70°C until they were used.

Purification of eosinophils

Eosinophils were obtained from the peritoneal cavity, as described by Mackenzie et al.¹⁵ Rats injected with 5 ml of saline solution intraperitoneally 2 or 3 days before cell isolation were killed by ether anesthesia, and the abdominal skin was cut open. Twenty milliliters of RPMI-1640 medium containing heparin, 20 U/ml, was injected into the peritoneal cavity. After gentle massage of the abdomen for 1 minute, the fluid was aspirated from the opened abdominal cavity. Cells obtained from three or four rats were pooled, washed, and then incubated in RPMI-1640 medium in 75 cm^2 ² plastic tissue-culture flasks (Corning Glass Works, Corning, N.Y.) for 1.5 hours at 37°C in air containing 5% CO_2 . Nonadherent cells were collected and resuspended in RPMI-1640 medium at a concentration of 3 to $8 \times 10^7/\text{ml}$ ⁷. One milliliter of the cell suspension was layered onto 4 ml of a discontinuous gradient of metrizamide (Cedarlane, Ontario, Canada), comprised of 2 ml of a 22.5% solution overlaid with 2 ml of a 18.5% solution, and centrifuged at room temperature for 15 minutes at 400 g . Three bands of cells resulted, and the second band mainly consisted of eosinophils (over 90%) with a small fraction of neutrophils (less than 10%).

Eosinophil chemotaxis

Eosinophils were suspended in RPMI-1640 medium at a concentration of $1 \times 10^6/\text{ml}$. In a 96-well chemotaxis chamber (Neuro Probe Inc., Cabin John, Md.), the upper compartment received 200 μl of cell suspension, and the lower compartment received 30 μl of RPMI-1640 medium or supernatant of cultured LN cells. A polyvinylpyrrolidone-free polycarbonate filter with 5 μm pores (Neuro Probe Inc.) separated the two layers. The entire chamber was incubated at 37°C for 2 hours in air containing 5% CO_2 , then the filter was removed and eosinophils that had migrated into the lower compartment were counted by use of cytopsin preparations subjected to May-Grünwald-Giemsa staining.

In vivo administration of anti-IL-5 antibody

Young rats were sensitized with ovalbumin as previously described. Rat monoclonal antibodies

against mouse IL-5, with a dose of 50 or 100 µg/body (Phar- Mingen, San Diego, Calif.) or equal volume of saline solution, were injected intraperitoneally 30 minutes after ovalbumin challenge. Two days after ovalbumin challenge, bronchoalveolar lavage was performed, and eosinophils were counted.

Assay of interferon- γ

The concentration of interferon- γ (IFN- γ) in LN cell culture supernatants was measured with an ELISA test kit (Gibco); the detection limit of this kit was 1 U/ml.

Analysis of lymphocyte subsets

Two days after ovalbumin challenge, lymphocytes were collected from bronchoalveolar lavage fluid (BALF) or popliteal LNs of sensitized rats with Ficoll-Paque ET (Pharmacia Biotech, Uppsala, Sweden).

Subsets of lymphocytes, CD4⁺ and CD8⁺, were analyzed by direct immunofluorescence methods with a cytofluorograph (Epics Profile II; Coulter Corp., Hialeah, Fla.). Briefly, 50 µl of lymphocyte suspension (2 to 5×10^5 cells) was incubated in the presence of saturating concentrations of fluorescein isothiocyanate–conjugated monoclonal antibodies in the dark on ice for 1 hour. Lymphocytes were washed twice with PBS containing 0.5% bovine serum albumin (Sigma). The number of immunofluorescence-positive cells per 5000 analyzed cells was determined. Specific binding of monoclonal antibodies was controlled by subtraction of mouse IgG₁–fluorescein isothiocyanate (Coulter Corp.). Monoclonal antibodies against CD4 and CD8 were purchased from Serotec (Kidlington, Oxford, U.K.).

Data analysis

Data are reported as mean $\pm$ SEM. Analysis of variance was used to analyze results from each age group over time. Differences between the two age groups and differences between the treated and control groups were analyzed by Student's unpaired *t* test. A *p* value of less than 0.05 was accepted as statistically significant.

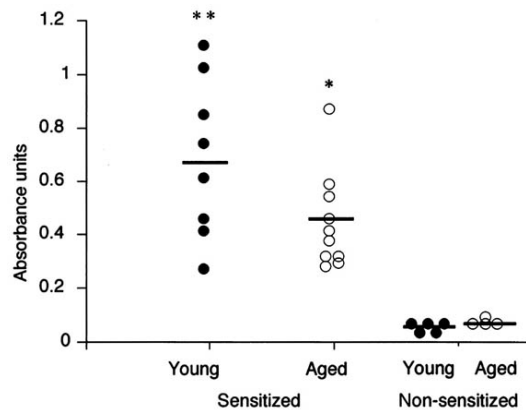
Results

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Ovalbumin-specific IgE production

There were no detectable levels of ovalbumin-specific IgE in the nonsensitized group of rats of either age (Fig. 1).

Fig. 1. Ovalbumin-specific IgE levels in serum from young and aged rats. Number of circles (*filled circles*, young; *open circles*, aged) indicates number of experiments. *Bars* indicate mean levels of each group. * $p < 0.01$ as compared with nonsensitized control group, ** $p < 0.01$ as compared with aged group.



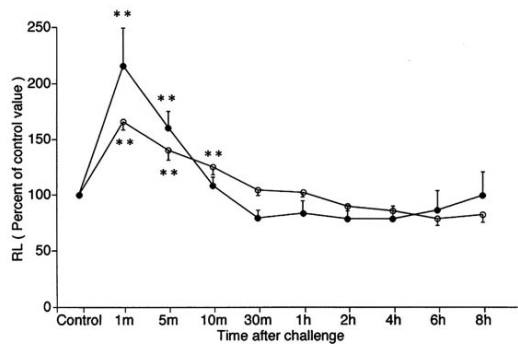
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Two weeks after sensitization, the young and aged groups each produced an ovalbumin-specific IgE antibody, but the level was significantly lower in the aged group than in the young group (young, 0.67 ± 0.28 vs aged, 0.41 ± 0.17).

RL after ovalbumin challenge

Both age groups exhibited a significant increase in RL immediately after exposure to ovalbumin, but the response returned to baseline within 10 to 30 minutes (Fig. 2).

Fig. 2. Time course of RL after ovalbumin challenge. Results are expressed as means $\pm$ SEM from six experiments (*filled circles*, young, $n = 6$; *open circles*, aged, $n = 6$). Rats were sensitized by subcutaneous injection of ovalbumin and challenged with ovalbumin aerosol. Both age groups exhibited a significant increase in RL immediately after exposure to ovalbumin. ** $p < 0.01$ as compared with control value.



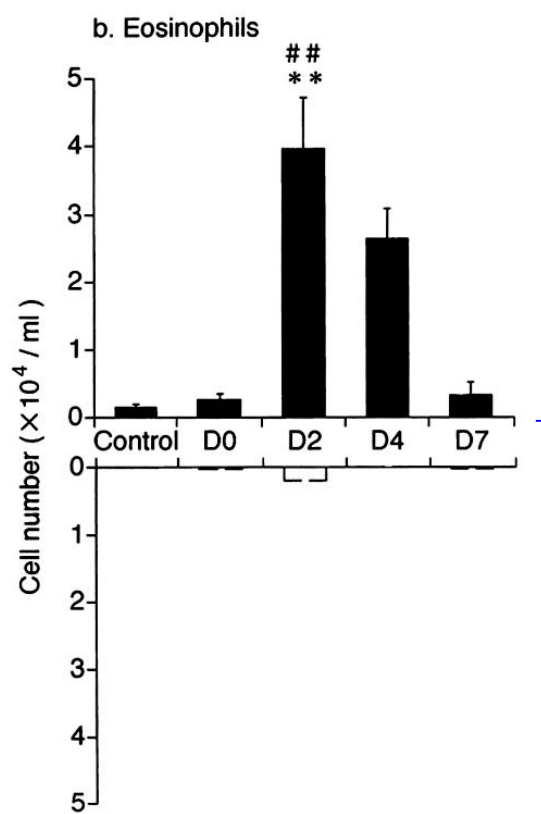
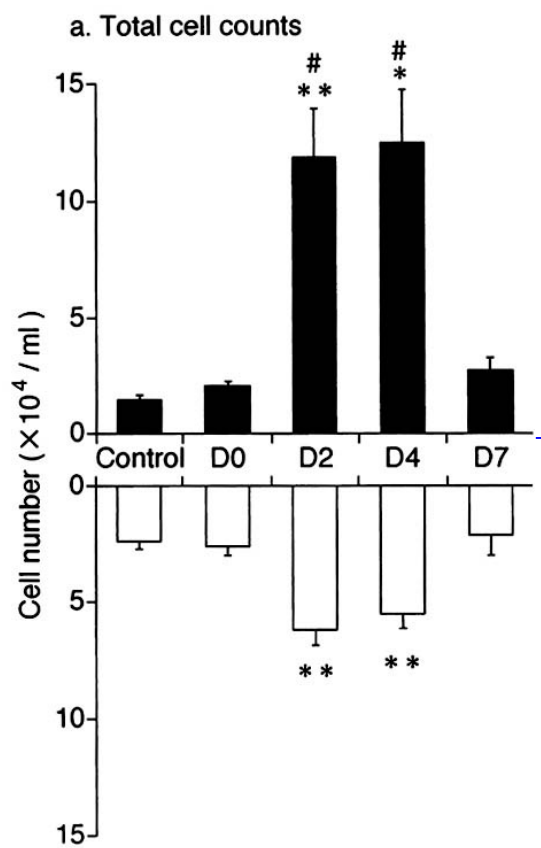
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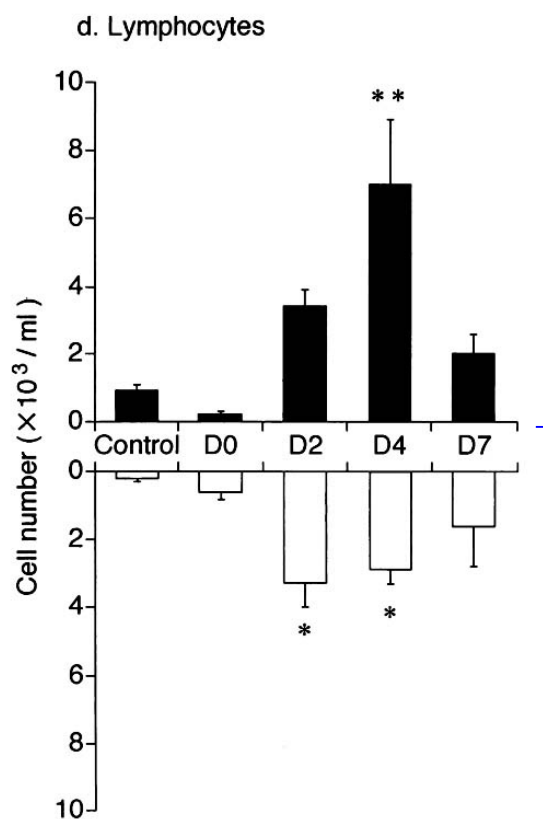
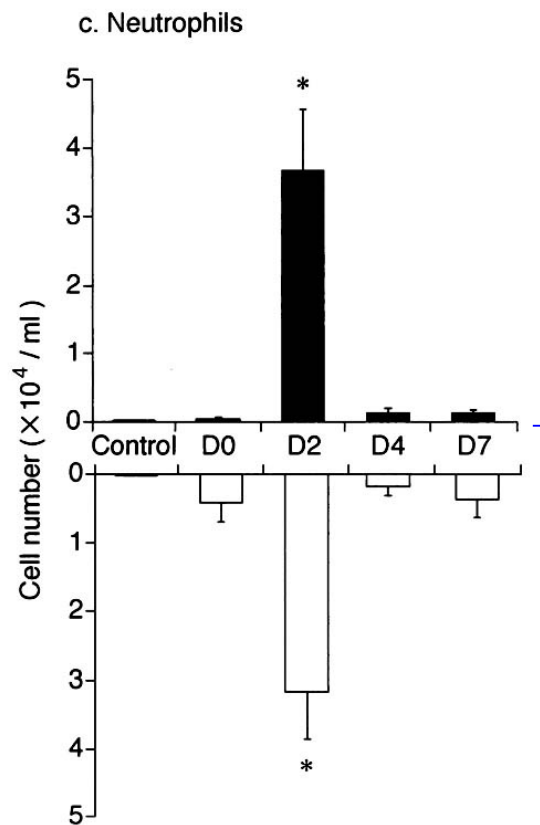
The degree of response appeared greater in young rats than in aged rats. A late bronchial reaction was not observed in either group during the 8-hour observation period.

Changes in cellular content of BALF

The time course of changes in BALF in young rats revealed that the influx of neutrophils was greatest 2 days after antigen challenge and had decreased greatly at 4 days after the challenge. In contrast, increases in the number of eosinophils in young rats persisted for several days (Fig. 3).

Fig. 3. Changes in cellular findings in BALF after exposure to ovalbumin. *Filled bar* indicates young rats ($n = 7$), and *open bar* indicates aged rats ($n = 7$). Eosinophil accumulation was not seen in the aged group after challenge (**b**). Control indicates results from nonsensitized rats. Results are expressed as means $\pm$ SEM. ** $p < 0.01$, * $p < 0.05$ as compared with control value. ### $p < 0.01$, # $p < 0.05$ as compared with aged rats.





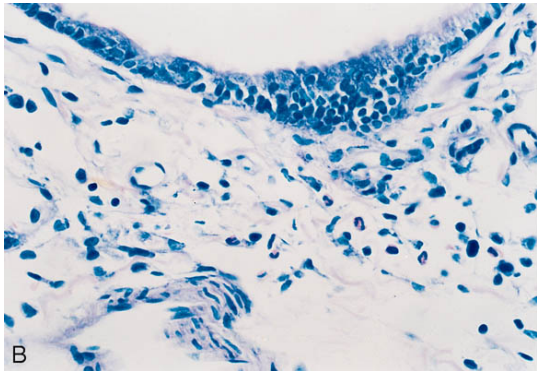
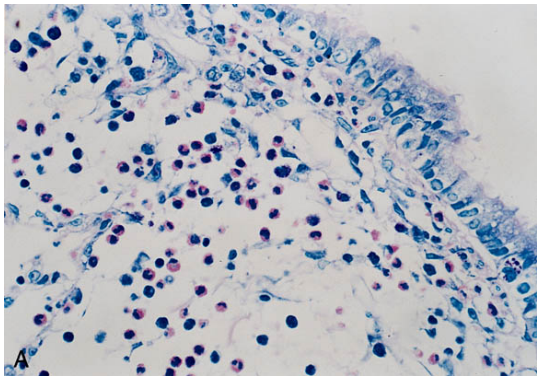
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Aged rats showed increases in the number of neutrophils equivalent to those of young rats. The number of lymphocytes was also equivalent to that in young rats 2 days after ovalbumin challenge, and the degree tended to be lower than that for young rats on the fourth day. In contrast, it was noted especially that exposure of aged rats to ovalbumin failed to evoke an increase in the number of eosinophils (Fig. 3 ■).

Histologic findings

A marked infiltration of the peribronchial regions with eosinophils and mononuclear cells was observed in lung sections from young rats 2 days after ovalbumin exposure (Fig. 4, A).

Fig. 4. Histologic findings 2 days after ovalbumin challenge. There is a marked infiltration of eosinophils into the subepithelial area in young rats **(A)**. In contrast, few eosinophils were observed in aged rats **(B)**.



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In contrast, only a small number of eosinophils was seen in lung sections from aged rats (Fig. 4, B ■).

The distribution of mast cells in the airways of normal and sensitized rats was similar in both age groups (Table I).

Table I. Distribution of mast cells in airways of young and aged rats						
	Trachea		Bronchus		Bronchiole	
	Epithelial	Subepithelial	Epithelial	Subepithelial	Epithelial	Subepithelial
Young						

Normal	+	+	++	+	-	+
Sensitized	+	+	++	+	-	+
Aged						
Normal	+	+	+	++	-	+
Sensitized	+	+	+	++	-	+

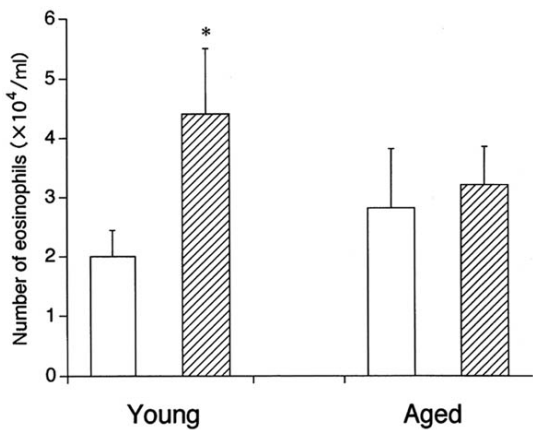
-, No mast cell; +, less than 4 mast cells in 250 µm of the epithelial or subepithelial area; ++, 4 or more mast cells in 250 µm of the epithelial or subepithelial area.

ECA in supernatant of cultured LN cells

To obtain further information about the failure of aged rats to exhibit an ovalbumin-induced accumulation of eosinophils, we examined ECA in the supernatant of ovalbumin-stimulated LN cells obtained from both age groups.

ECA was detectable in the supernatant of LN cells from the young rats, but not in that from the aged rats (Fig. 5).

Fig. 5. ECA of LN cell culture supernatant from young and aged rats. Results are expressed as mean eosinophil count in the bottom compartment, as determined by a modified Boyden chamber method. *Hatched bar*, Supernatant cultured with ovalbumin (*n* = 6); *open bar*, supernatant cultured without ovalbumin (*n* = 6), **p* < 0.01 as compared with control.



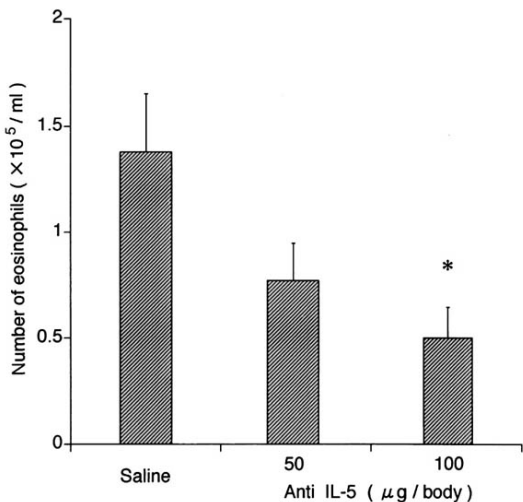
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In vivo treatment with anti-IL-5 antibody

We used a neutralizing antibody to IL-5 to investigate the nature of ECA. Pretreatment with rat anti-

mouse IL-5 monoclonal antibody inhibited eosinophil infiltration in a dose-dependent fashion in the BALF (Fig. 6).

Fig. 6. Inhibition of aeroallergen-induced infiltration of eosinophils in BALF of young rats by anti-IL-5 monoclonal antibody (*n* = 5). Administration of 100 µg of antibody significantly inhibited the eosinophil infiltration as compared with control (**p* < 0.05).



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IFN-γ production

To exclude the possibility that LN cells from aged rats had not been sensitized with ovalbumin, we measured IFN-γ levels in supernatants from LN cells that had been cultured with ovalbumin; we had observed earlier that the capacity of LN cells from aged rats to produce IFN-γ in response to concanavalin A or some other antigenic stimuli was well conserved (data not shown). The concentration of IFN-γ in supernatants of cultured LN cells stimulated with ovalbumin tended to be higher in aged rats than in young rats (6.5 ± 4.2 vs 2.4 ± 1.6 U/ml), but the difference was not significant. We could not detect IFN-γ in supernatants of nonstimulated cultured LN cells in either age group (Table II).

Table II. Concentration of IFN-γ in supernatant of cultured LN cells

	No. of experiments	IFN-γ (U/ml)
Young		
OA (+)	5	2.36 ± 0.79*
OA (-)	4	0.05 ± 0.03
Aged		

OA (+)	5	6.48 ± 2.09*
OA (-)	4	0.05 ± 0.03

Values are expressed as means ± SEM.

OA (+), Cultured with ovalbumin; OA (-), cultured without ovalbumin.

* $p < 0.01$ as compared with the OA (-) group.

Subsets of lymphocytes

We also investigated CD4⁺ and CD8⁺ T-cell profiles in popliteal LNs and BALF from ovalbumin-sensitized rats. In LNs from ovalbumin-sensitized rats, CD4⁺ T cells predominated as compared with CD8⁺ T cells in both age groups, and the value was significantly higher in young rats. Although CD4⁺ T cells also predominated in BALF, the degree was equivalent in both age groups (Table III).

Table III. CD 4/8 ratio in popliteal LN and BALF from ovalbumin-sensitized rats

	<u>CD 4/8</u>	
	Young ($n = 4$)	Aged ($n = 4$)
LN	13.3 ± 1.1	9.8 ± 1.4*
BALF	2.5 ± 0.5	3.0 ± 0.5

Values are expressed as means ± SEM.

* $p < 0.05$ as compared with the young group.

Discussion

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In our rat model of bronchial asthma, antigen-specific IgE production was induced after ovalbumin sensitization in both the young and aged animals. However, the levels of ovalbumin-specific IgE were lower in the aged rats than in the young rats. Both the serum IgE concentration and antigen-specific IgE formation decline with age in human beings^{6 8} and in experimental animals.^{16 17}

Pauwels et al.¹⁷ reported that the IgE response increased after birth, became a maximal at 2 months, then decreased progressively with age in Brown-Norway rats that were immunized with ovalbumin. Our results are in agreement with those findings. The analysis of lymphocyte subsets indicated the low value of the CD4/8 ratio in LNs from aged rats, which is thought to be associated with low concentration of antigen-specific IgE because CD4⁺ T cells play an important role in IgE synthesis¹⁸ and CD8⁺ T cells are potent suppressors of the primary IgE response.¹⁹

We found that exposure to ovalbumin produced an immediate bronchial response in rats in both

age groups. Such a response is induced by IgE-mediated mast cell activation. It appeared that the degree of bronchial response in aged rats was less than that in young rats. Histologic findings revealed a similar distribution and number of mast cells in the airways of both groups. Thus although a possible age-related alteration in the function of mast cells remains to be excluded, it seems likely that structural differences in the airway, including a wide airway caliber, reduced the airway compliance²⁰ and decreased the contractile ability of smooth muscle,^{21 22} contributing to the decrease in the bronchial response observed in aged rats. Another possible cause of decreased bronchial response is the low specific IgE antibody production in aged rats. However, there have been some reports describing the lack of correlation between specific IgE in the serum and respiratory response.^{23 24}

In our model a late bronchial reaction after ovalbumin inhalation was not observed in either age group, which differed from the findings of Eidelman et al.²⁵ This discrepancy may be due to the different methods used for sensitization, including the dose of ovalbumin, 1 mg (their experiment) versus 50 µg (ours), and the site of sensitization: back of the neck versus the foot pad, respectively. The optimal dose of antigen for inducing IgE antibody formation is reportedly between 10 and 1000 µg in Brown-Norway rats.²⁶ On the basis of that report, we determined, in a preliminary experiment, that young rats sensitized with 50 µg showed a greater bronchial response immediately after ovalbumin challenge than did rats given 1000 µg. We then chose the small dose for use in this study.

Aged rats failed to show an accumulation of eosinophils in the lungs after ovalbumin challenge, as determined by BALF and histologic examination. To investigate the mechanism of this reduced infiltration of eosinophils, we examined the capacity of the popliteal LN cells to produce ECA and found ECA to be present in the supernatant of cultured LN cells from young rats, but not in that from the aged rats. Results suggest that the mononuclear cells accumulated in the lungs in aged rats are also defective with respect to generation of ECA. Further examination by using neutralizing antibody, anti-IL-5 monoclonal antibody, revealed that IL-5, at least partially, caused antigen-induced eosinophil recruitment in the airways of young rats. It is reported that IL-5 is one of the key cytokines responsible for accumulation of eosinophils in the allergic inflammatory sites in experiments with mice,^{27 28} guinea pigs,²⁹ and human beings.^{30 31}

When we measured the concentration of IFN-γ in cultured LN supernatant to confirm the development of ovalbumin sensitization in aged rats, we found the concentration to be higher in the aged rats than in the young animals. Other investigators have also reported an increase in IFN-γ production by splenic lymphocytes of aged rodents after nonspecific or antigenic stimulation.^{11 12 32}

It is reported that IFN-γ regulates antigen-induced eosinophil recruitment into the mouse airway by inhibiting CD4⁺ T-cell infiltration.³³ There is a possibility that increased production of IFN-γ inhibited eosinophil accumulation after ovalbumin challenge in our aged rats. But the number of lymphocytes and the CD4/8 ratio in BALF after antigen challenge were not different between the two age groups. This indicates that the function of T cells, including the alteration of subgroup of CD4⁺ T cells in aged rats, may play a role in age-related changes in eosinophil accumulation.

Different patterns of cytokine release are characteristic of certain subgroups of T helper cells,

termed TH1 and TH2; the former produces IL-2 and IFN- γ , and the latter produces IL-3, IL-4, and IL-5 according to observations in mice.³⁴ IL-4 has been shown to be essential for inducing IgE synthesis;^{18 35} , and IFN- γ , which is considered to be the key cytokine for generating TH1-type cells, can inhibit some of the actions of IL-4.³⁶ Anti-rat IL-3, IL-4, and IL-5 antibodies were not commercially available, so we could not measure these cytokines directly. But the findings in this study—a decreased ovalbumin-specific IgE production, failure of eosinophil accumulation, and higher levels of IFN- γ production by ovalbumin-stimulated LN cells—suggest that cytokine profiles induced by ovalbumin stimulation in aged rats are shifted to those seen in TH-involved inflammation, whereas in young animals exposure to ovalbumin produced a TH2-involved allergic inflammation.

The matter of age-related alterations in lymphokines is complex. For example, not only IFN- γ but also IL-4^{11 12 37 38} , and IL-5¹¹ reportedly increase with age in the spleen cells of mice stimulated by nonspecific stimulants such as anti-CD3 monoclonal antibody, phorbol myristate acetate, and calcium ionophore. Age-related changes in IL-2 and IL-3 in mice remain controversial.^{10 11 38 39} Production of IL-4 and IFN- γ in human peripheral blood mononuclear cells is reported to decline with age when concanavalin A⁴⁰ is used for stimulation, a finding that suggests the existence of species differences in age-related changes in cytokine profiles. Although we did not exclude possible eosinophil dysfunction in aged animals, including the expression of adhesion molecules and the quantity and quality of cytokine receptors, our data indicate that the age-related alteration in cytokine profiles is an important complex matter that warrants further investigation. In particular, age-related alteration in cytokine profiles after specific antigen exposure is quite unknown, and experiments will be undertaken to clarify these points.

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Publishing and Reprint Information

TOP

- *From the Second Department of Internal Medicine, Hamamatsu University School of Medicine.*
- *Received for publication May 3, 1995.*
- *Revised Dec. 26, 1995.*
- *Accepted for publication Feb. 9, 1996.*
- *Reprint requests: Takesi Yagi, MD, Shimada Municipal Hospital, 1200-5 Noda, Shimada, 427, Japan.*
- **1/1/73107**
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