

Phenotypic comparison of allergic airway responses to house dust mite in three rat strains

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Singh, Pramila, Mary Daniels, Darrell W. Winsett, Judy Richards, Donald Doerfler, Gary Hatch, Kenneth B. Adler, and M. Ian Gilmour. Phenotypic comparison of allergic airway responses to house dust mite in three rat strains. *Am J Physiol Lung Cell Mol Physiol* 284: L588–L598, 2003. First published November 22, 2002; 10.1152/ajplung.00287.2002.—Brown Norway (BN) rats develop a robust response to antigens in the lung, characterized by a large increase in allergen-specific immune function and pulmonary eosinophilia. The objective of this study was to investigate alternative models by determining whether other rat strains could be sensitized to house dust mite (HDM) antigen and whether the allergic disease process could be worsened with repeated allergen exposure. In general, BN rats sensitized by either subcutaneous or intratracheal routes exhibited increased pulmonary allergy compared with Sprague-Dawley (SD) and Lewis (L) rats. Multiple intratracheal allergen exposures incrementally increased HDM-specific immune function in BN rats but progressively decreased eosinophil recruitment and markers of lung injury. SD rats had more moderate responses, whereas L rats were relatively unresponsive. Because BN rats developed stronger clinical hallmarks of allergic asthma under various immunization regimes compared with SD and L rats, we conclude that the BN is the most appropriate strain for studying allergic asthma-like responses in rats. Phenotypic differences in response to HDM were associated with differences in the Th1/Th2 cytokine balance and antioxidant capacity.

genetic variability; atopy; asthma

ANIMAL MODELS of allergic airway disease are useful tools for studying susceptibility factors and pathophysiological processes associated with human allergic asthma. Numerous genetic differences among rat and mouse strains in IgE responsiveness and other phenotypes of respiratory allergy have been reported. Studies in allergy-susceptible (A/J strain) and allergy-resistant (C3H strain) mice suggest that the genes associated with a Th2-type cytokine profile and its regulation by other cytokines, like IL-12 and IFN- γ , may determine phenotypic responses to allergen challenge (12, 31). Brown Norway (BN) rats have consis-

tently demonstrated high IgE responsiveness to allergen provocation and significantly greater constitutive IgE production (22, 26) compared with other rat strains (28). BN rats are also high responders in pulmonary pathologies associated with parainfluenza virus infection (25), although both Lewis (L) and Sprague-Dawley (SD) rats have demonstrated susceptibility to experimentally induced airway hyperresponsiveness (18) as well as experimental allergic neuritis (9) and neurogenic inflammation (11). It would then seem likely that all three rat strains would have potential for use in models of inflammatory and allergic airway diseases. However, studies of ovalbumin (OVA)-induced respiratory allergy have shown that L and SD rats do not develop pulmonary eosinophilia (21) and airway hyperresponsiveness (3) following OVA challenge. Pulmonary responsiveness to house dust mite (HDM) antigen has not been compared among these three rat strains, and an integrated assessment of immune responses, altered pulmonary function, and pathological processes would create a more complete view of the similarities and differences among the three phenotypes.

The tendency toward high baseline responses in untreated BN rats and interindividual variability in the intensity of allergic responses to allergen challenge are two recurrent problems that can confound investigations of allergic pathophysiology. This paper examines whether or not a different rat strain could provide an alternative model of human allergic airway disease with lower baseline levels and less variability than observed in BN rats. Because bronchoconstriction, eosinophilic inflammation of the airways, and mucus production are three of the hallmarks of human allergic asthma, these responses should be included in an animal model of the disease. In addition, although decreased plasma levels and dietary deficiencies in ascorbic acid (vitamin C) have been associated with asthma (7), it is not known whether strain-dependent variations in antioxidant capacity affect susceptibility to allergic sensitization. The present study tested the hypothesis that the BN is the most appropriate rat

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strain for investigating allergic airway disease and asthma because of its pro-Th2 and proeosinophilic phenotype. We compared response profiles among BN, L, and SD rats sensitized either subcutaneously (SC) or intratracheally (IT) to better understand associations among lung injury and inflammation, immune function, and a key symptomatic indicator of disease, allergen-induced bronchoconstriction. Additionally, we investigated the effect of multiple allergen challenges to determine whether immunological tolerance or chronic disease would develop. This integrated evaluation of pulmonary allergy included measurements of immediate airway responses (IARs) to HDM challenge and allergen-specific serum IgG and IgE levels, HDM-specific proliferation of lymphocytes in bronchial lymph nodes, antioxidant levels, inflammatory cell infiltrate, and IL-13 (Th2 cytokine) and IFN- γ (Th1 cytokine) concentrations in bronchoalveolar lavage fluid (BALF).

MATERIALS AND METHODS

Animals. Inbred, female BN (strain BN/Ss Nhsd; 160–180 g); inbred, female L (210–240 g); and outbred, female SD (250–280 g) rats were purchased from Charles River Laboratories (Wilmington, MA) and used at 8–10 wk of age. Rats were housed in American Association for Accreditation of Laboratory Animal Care-approved animal facilities with high-efficiency particulate air filters, and their use was reviewed by the U. S. Environmental Protection Agency's Animal Care and Use Committee. All rats were fed rat chow and water ad libitum. All rats that were selected randomly and serologically tested upon arrival, as well as sentinels monitored throughout the study, were free of Sendai virus, pneumonia virus, and a variety of other rodent viruses and *Mycoplasma* sp.

Antigen. HDM antigen derived from *Dermatophagoides farinae* (*Der f*) was purified from ground, whole-bodied mites following defatting, extraction in 0.125 M ammonium bicarbonate, and dialysis with distilled water (Greer Laboratories, Lenoir, NC). Purification of the extract was achieved by a combination of DEAE ion exchange chromatography and gel filtration, with the final preparation containing >75% of group 1 allergen (*Der f* 1), as determined by the vendor.

Experimental design. BN, L, and SD rats were sensitized with HDM by either systemic (SC) or local (IT) routes. Systemic injection was assessed for maximal immunization; however, we were also interested in mucosal sensitization as a more relevant exposure route and because of the potential for different sensitization regimes to produce different immunological effects. IT instillation is a quick, easily reproducible procedure and shows results comparable with those based on the inhalation route of administration (8). On *days 1* and *3* systemically sensitized rats received an SC injection of 5 μ g of HDM in 0.5 ml of aluminum hydroxide adjuvant (Alhydrogel containing 1.3% Al₂O₃ as dry matter; Accurate Chemical & Scientific, Westbury, NY) or 0.5 ml of aluminum hydroxide adjuvant alone as a sham sensitization. Two weeks later these rats were challenged IT with 10 μ g of HDM in 250 μ l of saline. Airway responses to HDM were measured on the day of challenge, and rats were euthanized and bled by cardiac puncture 2 days later for assessment of allergic responses, pulmonary inflammation, and lung injury in tissue samples. Postchallenge *day 2* was selected as the time point for evaluation of responses to allergen, because it is known that pulmonary inflammation and injury peak 2–3 days postchallenge (21). Locally sensitized BN, L, and SD rats received 5

μ g of HDM in 250 μ l of saline by IT instillation on *days 1* and *3*. Two weeks later, separate groups of these rats were IT challenged with 10 μ g of HDM in 250 μ l of saline either one, two, or five times, each successive challenge separated by 1 wk. Airway responses to HDM were evaluated in all locally sensitized rats on the last day of allergen challenge. These rats were euthanized and bled by cardiac puncture 2 days after the final IT challenge for assessment of allergic responses, pulmonary inflammation, and lung injury.

IARs to HDM. Two weeks after sensitization, animals were placed in a whole body plethysmograph (Buxco Electronics, Troy, NY) equipped with a pneumotachograph and pressure transducer to monitor pulmonary ventilation responses as previously described (15–17). Baseline ventilatory readings were measured for 10 min before challenge. Animals were then removed from the plethysmograph, anesthetized with halothane, IT instilled with 10 μ g of HDM in 250 μ l of saline, and then placed in the plethysmograph for an additional 20 min to evaluate IARs following challenge. These responses are expressed as the enhanced pause (Penh), which is a derived value that provides an indicator of changes in specific airway resistance (20). Penh values were averaged during the baseline (control) periods and the postchallenge periods to obtain mean values for each event and are represented as change from the mean during the baseline period to the mean during the postchallenge period.

Bronchoalveolar lavage and lavage cell differentials. The trachea of each rat was surgically exposed, cannulated, and tied off with a silk thread suture. The left lobe was tied off to prepare for histopathology, and the right lobe was lavaged three times with a single volume of warmed saline ($0.035 \times$ body wt $\times 0.55$ ml/kg). Total white blood cell counts were obtained with a hemocytometer, and cell viability was assessed by trypan blue exclusion. Approximately 50,000 cells from each sample were centrifuged onto duplicate glass slides in a Cytospin (Shandon, Pittsburgh, PA) and stained with Diff Quik (American Scientific, Sewickley, PA) for identification of eosinophils, macrophages, neutrophils, and lymphocytes. We counted at least 200 cells for each duplicate slide to obtain percent values of each leukocyte subpopulation. If the difference between duplicate slides was >2%, then the slides were recounted until agreement in the differential was reached.

BALF biochemical analysis. BALF was centrifuged (1,500 rpm, 10 min, 4°C), and the supernatant was analyzed. Lactate dehydrogenase (LDH) and total protein levels were determined with kit 228 (Sigma) and Coomassie Plus reagent (Pierce, Rockford, IL), respectively. We adapted both assays for automated analysis using a Cobas Fara II centrifugal spectrophotometer (Hoffman-La Roche, Branchburg, NJ). Perchloric acid (PCA) was added to a separate aliquot of BALF for antioxidant analysis at a final concentration of 3%, and samples were stored at –80°C. Before analysis for reduced glutathione (GSH), ascorbic acid (AA), uric acid (UA), and nonprotein sulfhydryls (NPSH), PCA-treated samples were centrifuged at 20,000 *g* for 30 min at 4°C (24). AA and UA analyses were performed by liquid chromatography with electrochemical detection as described in Kutnink et al. (14), and total GSH was measured in the presence of GSH reductase and 5,5'-dithiobis-(2-nitrobenzoic acid) by enzymatic recycling (1) by a Cobas Fara II centrifugal spectrophotometer. The detection limit for both AA and UA was 0.2 nmol/ml. NPSH concentrations in BALF were determined with a reagent containing 2.0 mM ethylene-diaminetetraacetic acid and 0.21 mM 5,5'-dithiobis-2-nitrobenzoic acid in a 0.4 M Tris·HCl buffer, pH 8.9 (27). We determined the sample concentration of NPSH from a standard curve using reduced

glutathione. The limit of detection for both GSH and NPSH was 0.21 $\mu\text{g/ml}$.

BALF cytokine analysis. An aliquot of BALF (stored at -80°C) was used for analysis of IFN- γ and IL-13 cytokines as representative markers of Th1 and Th2 phenotypes, respectively. We assessed concentrations by enzyme-linked immunosorbent assay (ELISA) using rat Cytoscreen kits purchased from Biosource International (Camarillo, CA). Selection of these cytokines was based upon IFN- γ 's recognition as the prototypic Th1 cytokine and the fact that IL-13 has been conclusively linked to the development of respiratory allergy (29, 30).

Lymphocyte proliferation assay. Lung-associated lymph nodes were removed from the right main stem bronchus of each rat 2 days after (final) HDM challenge. We prepared single cell suspensions of lymph node tissues using ground-glass homogenizers, and the in vitro lymphocyte proliferation response to HDM was assessed by cellular incorporation of [^3H]thymidine as described in Gilmour et al. (5). Allergen-specific lymphocyte proliferation was expressed as the difference in radiotracer counts (disintegrations per min) between lymphocytes from a given sample incubated with HDM (1 $\mu\text{g/well}$) and lymphocytes from the same sample incubated with media alone.

Antigen-specific serum IgE and serum IgG. Antigen-specific serum immunoglobulin production was measured by ELISA as described previously (5). Rats were euthanized with 200 mg/kg pentobarbital sodium and bled by cardiac puncture. Serum was prepared and kept frozen at -80°C until assay. Briefly, for the HDM-specific IgE assay, 96-well flat-bottom ELISA plates were coated with 100 $\mu\text{l/well}$ of mouse anti-rat IgE heavy chain antibody (Serotec, Oxford, UK) at a concentration of 2.5 $\mu\text{g/ml}$ in coating buffer (Pierce) and incubated overnight at 4°C . The following day, after a blocking step and washing, 100 μl of each serum sample (diluted 1:5 in blocking buffer) were added in duplicate wells to the plates. After an overnight incubation at 4°C and washing, the plates were treated successively with 100 $\mu\text{l/well}$ of biotinylated HDM (2 $\mu\text{g/ml}$, prepared using Sulfo-NHS-LC-Biotinylation kit; Pierce) and horseradish peroxidase-streptavidin (diluted 1:1,500), with washes and incubation for 1 h at room temperature between each of these steps. For the HDM-specific serum IgG assay, 96-well flat-bottom plates were coated with 100 μl of purified HDM (containing $>75\%$ *Der f* 1 antigen) at a concentration of 1.6 $\mu\text{g/ml}$ in coating buffer and incubated overnight at 4°C . The next day, after washing, 200 μl of blocking buffer were added to each well followed by a 2-h incubation at 37°C . Diluted serum (1:50) was added (100 $\mu\text{l/well}$) after washing followed by overnight incubation at 4°C . Wells were then washed and treated successively with 100 μl of mouse anti-rat biotinylated IgG (diluted 1:5,000; Serotec) and horseradish peroxidase-streptavidin (diluted 1:1,500) separated by washes and an incubation for 1.5 h at 37°C . In the final step, 100 $\mu\text{l/well}$ TM Blue (Dako, Carpinteria, CA) was added as a substrate for horseradish peroxidase, and reactions were allowed to develop at room temperature for at least 10 min. Plates were read at 650 nm by a Spectromax ELISA plate reader (Molecular Devices, Menlo Park, CA).

Histopathology. The left lobe of the lung was inflated and fixed with a volume ($0.035 \times \text{body wt} \times 0.40 \text{ ml/kg}$) of paraformaldehyde (4% wt/vol in deionized water) (Sigma), immersed in 4% paraformaldehyde for 24 h then transferred into Tris-buffered saline (pH 7.4) (Pierce). Samples were then sent to Experimental Pathology Laboratories (Research Triangle Park, NC) for processing and histopathological evaluation. Midsagittal lung sections were stained with hematox-

ylin and eosin to determine inflammatory changes and periodic acid Schiff/Alcian blue (pH 2.5) to determine goblet cell hypertrophy and hyperplasia and mucus production. Lung sections were microscopically evaluated in seven different histopathological lesion categories by open examination (10). These categories included perivascular lymphocytic infiltration, terminal bronchiolar lymphocytic infiltration, peribronchiolar eosinophilic infiltration, terminal bronchiolar eosinophilic infiltration, perivascular eosinophilic infiltration,

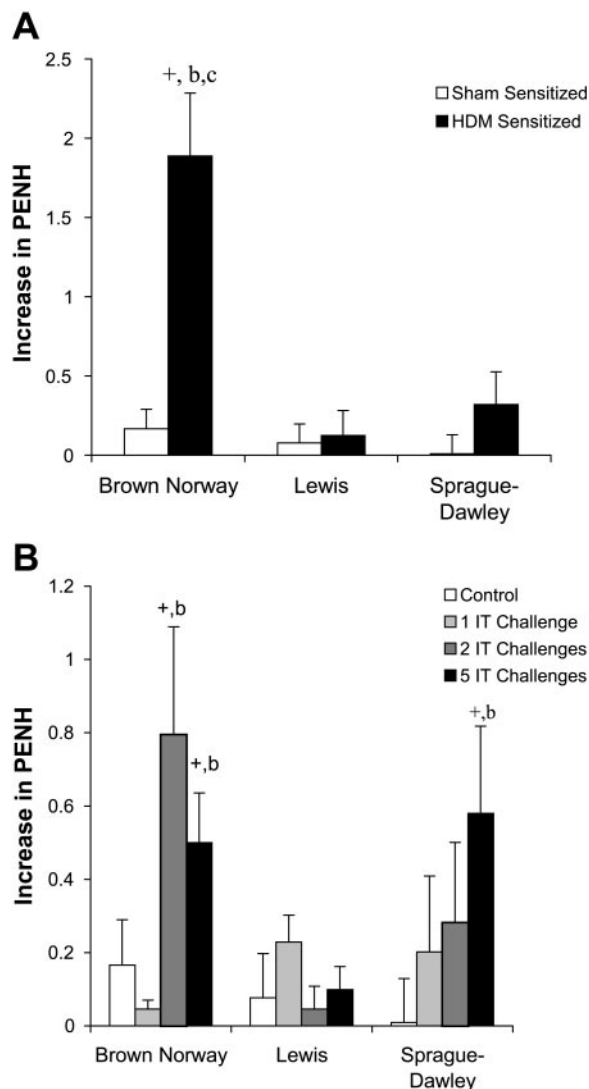


Fig. 1. Immediate airway responses (IARs) to house dust mite (HDM) challenge [10 μg of HDM in 250 μl of saline given intratracheally (IT)] over a period of 20 min following instillation. IARs in systemically [subcutaneously (SC) A] or locally (B) sensitized rats on the day of challenge and immediately after IT challenge with HDM. Baseline ventilatory readings were measured for 10 min before challenge in each rat. Responses are expressed in arbitrary units of enhanced pause (Penh). Data are presented as means $\pm$ SE for each treatment group ($n = 4-6$) and were analyzed by either 1-way (by strain) or 2-way (by strain and treatment) ANOVA, followed by pairwise comparisons among strains and various strain-treatment combinations. Significance was determined at $P < 0.05$. ⁺Significantly different from sham-sensitized treatment group within strain; ^bsignificantly different from L rats that received the same treatment; ^csignificantly different from SD rats that received the same treatment.

focal/multifocal pneumonitis, and goblet cell hypertrophy/hyperplasia with mucus in the airways. In each of these seven different categories, lesions were scored for both severity of inflammatory cell infiltrate and distribution: 0 (normal), 1 (minimal), 2 (mild), 3 (moderate), or 4 (marked). We derived the total lesion scores (Fig. 8) by adding up the combined lesion scores (0–4) from all seven categories for each sample and calculating the mean total lesion score for each treatment group.

Statistical analysis. The data were analyzed (SAS version 6.02; SAS Institute, Cary, NC) by analysis of variance (ANOVA) models. We used one-way (by strain) ANOVA for the locally sensitized groups and two-way (by strain and treatment) ANOVA for the systemically sensitized groups. After an overall statistically significant finding, pairwise comparisons were performed among strains and various strain-treatment combinations. Significance levels were adjusted for multiple comparisons by a Bonferroni technique. The level of significance was set at ≤ 0.05 .

RESULTS

IARs. After systemic immunization and challenge, only the BN rats developed a strong IAR compared with sham-immunized, challenged controls (Fig. 1A). With local sensitization, BN rats that were challenged two or more times developed significant IARs, although these responses were not as strong as with systemic immunization. Local immunization also resulted in the development of a significant IAR in SD rats after five challenges, which was of the same magnitude as the BN response. L rats did not develop IARs under any conditions.

BALF cell differentials. Among systemically sensitized rats, there were no significant strain differences

in numbers of macrophages, polymorphonuclear cells (PMNs)/neutrophils, or lymphocytes in the BALF (Table 1). However, there were much higher numbers of eosinophils in the BALF of both control and antigen-challenged BN rats compared with the same groups of L and SD rats on postchallenge day 2 (Fig. 2A). Systemic sensitization to HDM significantly increased total numbers of eosinophils in BALF (11-fold higher) over sham sensitization in BN rats (Fig. 2A).

After local sensitization, total numbers of eosinophils were significantly boosted in BN rats compared with L and SD rats after one, two, and five IT challenges, although multiple challenges dramatically diminished the eosinophilia (Fig. 2B). BN rats had significantly greater numbers of BALF macrophages than SD rats following two and five IT challenges and significantly more macrophages than L rats after five IT challenges (Table 1). There were no significant strain differences in numbers of PMNs in the BALF following one, two, and five challenges. In general, local sensitization induced greater inflammatory cell influx than systemic sensitization in all three strains, and a progressive reduction in eosinophil, macrophage, PMN, and lymphocytes was observed with increasing IT challenges (Table 1).

BALF biochemical analysis. All three strains demonstrated significant increases in total protein levels postchallenge in the BALF compared with their sham-sensitized controls (Fig. 3A). Systemically sensitized BN and SD rats had significantly greater amounts of total protein in the BALF on postchallenge day 2 than L rats that received the same treatment (Fig. 3A).

Table 1. Differential cell influx into BALF 2 days after final IT challenge with HDM

Rat Strain	Sensitization Route	Treatment	Cell Count $\times 10^4/\text{ml}$				
			Total	Eosinophils	Macrophages	Neutrophils (PMNs)	Lymphocytes
BN	Systemic	Sham	38.8 ± 9.5	2.45 ± 1.65	34.76 ± 9.05	0.36 ± 0.29	1.22 ± 0.90
		HDM	68.9 ± 12.1	$33.81 \pm 8.93^{*†‡}$	32.31 ± 5.02	$2.04 \pm 1.29^*$	0.78 ± 0.34
	Local	1 IT	$146. \pm 78.8$	$91.37 \pm 64.76^{*†‡}$	47.30 ± 12.75	$4.19 \pm 1.37^*$	$3.50 \pm 1.43^\dagger$
		2 IT	93.2 ± 21.0	$53.74 \pm 18.01^{*†‡}$	$27.74 \pm 4.46^\ddagger$	8.26 ± 4.12	$3.42 \pm 1.27^\dagger$
		5 IT	22.6 ± 2.3	$2.66 \pm 0.83^\ddagger$	$19.69 \pm 1.52^\ddagger$	0.15 ± 0.06	0.19 ± 0.05
L	Systemic	Sham	12.5 ± 2.0	0.17 ± 0.09	10.84 ± 2.82	0.03 ± 0.03	0.06 ± 0.04
		HDM	18.3 ± 3.5	0.30 ± 0.17	17.96 ± 3.57	0.00 ± 0.00	0.06 ± 0.06
	Local	1 IT	55.0 ± 19.4	2.49 ± 1.41	39.98 ± 9.29	11.57 ± 10.17	0.60 ± 0.28
		2 IT	16.7 ± 2.7	0.57 ± 0.23	15.98 ± 2.54	0.04 ± 0.04	0.07 ± 0.04
		5 IT	12.1 ± 1.8	0.02 ± 0.02	11.97 ± 1.80	0.04 ± 0.02	0.05 ± 0.04
SD	Systemic	Sham	17.4 ± 3.7	0.84 ± 0.52	18.34 ± 4.13	0.07 ± 0.07	0.26 ± 0.17
		HDM	19.3 ± 4.1	0.54 ± 0.29	17.33 ± 3.77	0.75 ± 0.72	0.71 ± 0.27
	Local	1 IT	35.0 ± 5.8	2.35 ± 0.65	28.96 ± 5.30	1.86 ± 1.25	$2.00 \pm 0.47^{*†}$
		2 IT	16.4 ± 2.9	0.71 ± 0.25	13.18 ± 3.01	1.09 ± 0.57	1.38 ± 0.54
		5 IT	13.0 ± 2.7	0.00 ± 0.00	12.16 ± 2.47	0.55 ± 0.36	0.25 ± 0.10

Total differential cell counts in bronchoalveolar lavage fluid (BALF), including eosinophils, macrophages, neutrophils, and lymphocytes, in rats either sham- or house dust mite (HDM)-sensitized by subcutaneous (SC) and intratracheal (IT) routes. Total cell counts and total cell subpopulation counts/ml BALF in systemically (SC) sensitized rats following a single IT challenge with HDM and in locally (IT) sensitized rats following 1, 2, or 5 IT challenges. Pulmonary inflammatory cells were obtained by BAL and counted by centrifuging BALF samples onto microscope slides and staining with Diff Quick. BALF samples were obtained 2 days after the final HDM challenge. Data are presented as means $\pm$ SE for each treatment group ($n = 4-6$). Data were analyzed by either 1-way (by strain) or 2-way (by strain and treatment) ANOVA, followed by pairwise comparisons among strains and various strain-treatment combinations. Significance was determined at $P < 0.05$. *Significantly different from sham-sensitized treatment group within strain. † Significantly different from Lewis (L) rats that received the same treatment. ‡ Significantly different from Sprague-Dawley (SD) rats that received the same treatment. BN, Brown Norway; PMN, polymorphonuclear neutrophils.

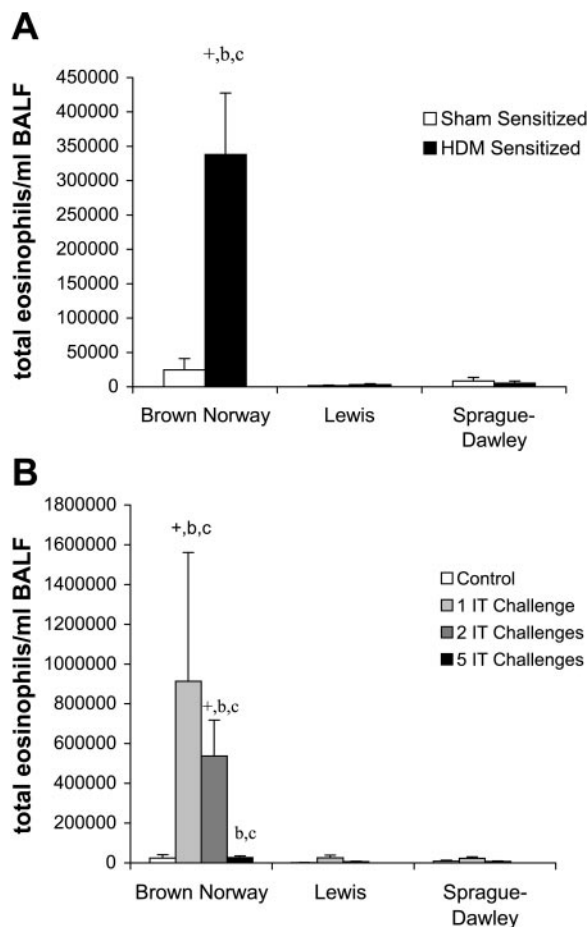


Fig. 2. Total eosinophil counts in bronchoalveolar lavage fluid (BALF) of rats either sham or HDM sensitized by SC and IT routes. Total eosinophil counts/ml BALF in systemically (SC, A) or locally (B) sensitized rats on postchallenge day 2, following IT challenge with HDM. Data are presented as means $\pm$ SE for each treatment group ($n = 4-6$) and were analyzed by either 1-way (by strain) or 2-way (by strain and treatment) ANOVA, followed by pairwise comparisons among strains and various strain-treatment combinations. Significance was determined at $P < 0.05$. ⁺Significantly different from sham-sensitized treatment group within strain; ^bsignificantly different from L rats that received the same treatment; ^csignificantly different from SD rats that received the same treatment.

There were no significant differences in levels of LDH in BALF of HDM-sensitized rats compared with their respective sham-sensitized controls in any strain (data not shown). However, the LDH levels of BN rats were significantly higher than those of SD and L rats with both systemic and local sensitization (data not shown). Among all locally sensitized rats, LDH (data not shown) and total protein (Fig. 3B) levels in BALF steadily decreased with increasing IT challenges. After five IT challenges, total protein levels in L rats dropped more than in BN and SD rats, accounting for a significant difference between BN and SD compared with L rats with this treatment (Fig. 3B).

Significant differences in BALF antioxidant levels were observed among the three rat strains. With systemic sensitization to HDM, SD rats had significantly higher levels of GSH in the BALF than BN or L rats

(Table 2). The amounts of BALF GSH in rats systemically sensitized with HDM were not statistically different from those of sham-sensitized rats, regardless of strain. There were no significant differences among the three strains in levels of NPSH, AA, or UA in the BALF with systemic sensitization; however, there was a trend in SD rats toward higher baseline levels of AA compared with L and BN rats (Table 2).

Locally sensitized L rats had significantly higher levels of GSH than BN and SD rats following two IT instillations of HDM (Table 2). GSH levels in the BALF were highest with only a single IT challenge in L and SD rats, whereas in BN rats, levels were consistently low with either one, two, or five IT challenges (Table 2). Concentrations of AA and UA were highest among all three rat strains following a single IT challenge. UA levels were significantly higher in BN rats compared with L rats, although they decreased after five chal-

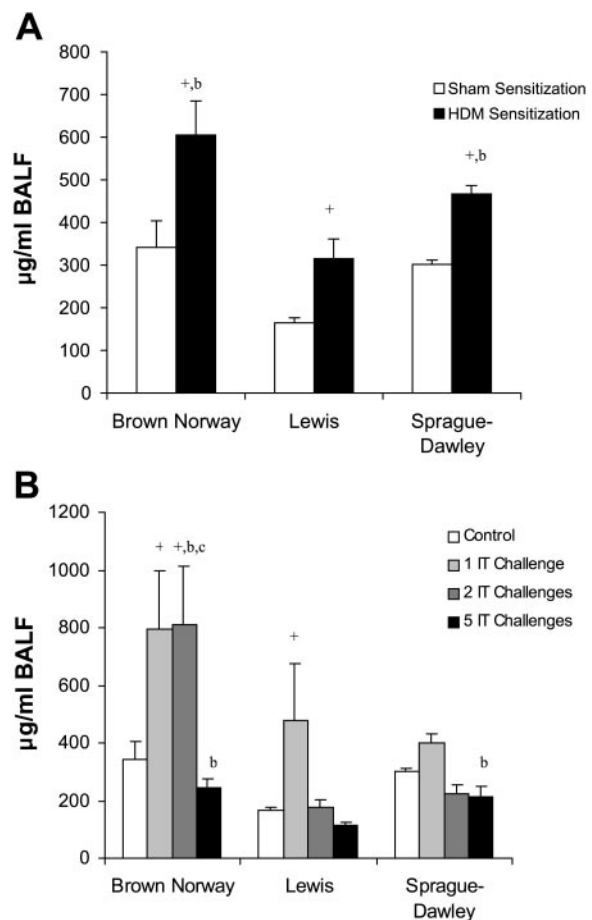


Fig. 3. Total protein in BALF of rats either sham or HDM sensitized by SC and IT routes. Total protein concentrations in systemically (SC) (A) and locally (B) sensitized rats on postchallenge day 2 following IT challenge with HDM. Data are presented as means $\pm$ SE for each treatment group ($n = 4-6$) and were analyzed by either 1-way (by strain) or 2-way (by strain and treatment) ANOVA, followed by pairwise comparisons among strains and various strain-treatment combinations. Significance was determined at $P < 0.05$. ⁺Significantly different from sham-sensitized treatment group within strain; ^bsignificantly different from L rats that received the same treatment; ^csignificantly different from SD rats that received the same treatment.

Table 2. Antioxidant concentrations in BALF 2 days after final IT challenge with HDM

Rat Strain	Sensitization Route	Treatment	Reduced Glutathione	Nonprotein Sulfhydryls	Ascorbic Acid	Uric Acid
BN	Systemic	Sham	0.34 ± 0.09	1.178 ± 0.418	2179.62 ± 356.12	608.95 ± 176.73
		HDM	0.38 ± 0.04	0.843 ± 0.343	1738.68 ± 588.69	685.93 ± 105.74
	Local	1 IT	0.414 ± 0.129	0.0 ± 0.0	1944.71 ± 323.38	1797.56 ± 660.15
		2 IT	0.242 ± 0.03	0.416 ± 0.197	1361.07 ± 234.41	1354.24 ± 438.09‡§
		5 IT	0.31 ± 0.03	1.032 ± 0.055	1148.7 ± 47.43‡	186.93 ± 39.35
L	Systemic	Sham	0.6 ± 0.17	0.503 ± 0.265	1729.06 ± 264.27	354.93 ± 96.06
		HDM	0.68 ± 0.23	0.244 ± 0.16	1436.1 ± 271.87	658.84 ± 102.46*
	Local	1 IT	4.362 ± 2.465	2.25 ± 1.29†§	2290.98 ± 646.29	998.31 ± 376.28
		2 IT	0.365 ± 0.046†§	0.368 ± 0.14	1074.1 ± 171.2	284.23 ± 54.24
		5 IT	0.25 ± 0.026	0.898 ± 0.227	615.54 ± 125.87	154.99 ± 151.59
SD	Systemic	Sham	2.98 ± 1.66	1.31 ± 1.08	3399.2 ± 1088.05	581.94 ± 237.43
		HDM	1.26 ± 0.65†‡	1.552 ± 0.83	2442.3 ± 529.31	869.98 ± 105.03*
	Local	1 IT	2.18 ± 0.683	0.0 ± 0.0	3187.09 ± 595.61	1246.43 ± 224.28
		2 IT	0.2 ± 0.019	0.372 ± 0.11	984.83 ± 202.08	314.01 ± 65.54
		5 IT	0.302 ± 0.038	1.724 ± 0.154†‡	856.0 ± 95.65	83.83 ± 40.48

Antioxidant levels in BALF of rats either sham- or HDM-sensitized by SC and IT routes. Reduced glutathione, nonprotein sulfhydryls, ascorbic acid, and uric acid concentrations ($\mu\text{g/ml}$) in systemically (SC) and locally (IT) sensitized rats on postchallenge day 2, following single or multiple IT challenge with HDM. Data are presented as means $\pm$ SE for each treatment group ($n = 4-6$) and were analyzed by either 1-way (by strain) or 2-way (by strain and treatment) ANOVA, followed by pairwise comparisons among strains and various strain-treatment combinations. Significance was determined at $P < 0.05$. *Significantly different from sham-sensitized treatment group within strain. †Significantly different from BN rats that received the same treatment. ‡Significantly different from L rats that received the same treatment. §Significantly different from SD rats that received the same treatment.

lenges. NPSH levels decreased with successive challenges in L rats, whereas they increased with successive challenges in BN and SD rats. Peak NPSH levels were highest in L and SD rats (Table 2).

To determine whether the HDM-induced changes in immune phenotype and lung injury could be attributed to differences in cytokine profiles, we assayed IFN- γ and IL-13 as markers of Th1 and Th2 products, respectively. Baseline IFN- γ tended to be higher in SD and L rats, whereas baseline IL-13 was higher in BN rats (Fig. 4A). In systemically immunized rats, challenge with HDM reduced IFN- γ in BN rats and to a lesser extent in SD rats but not in L rats. With the same immunization, challenge increased IL-13 in BN rats but did not have this effect in L or SD rats. In locally immunized animals, IFN- γ was significantly decreased in BN rats, whereas this suppression was less marked in L or SD rats and required multiple challenges (Fig. 4B). IL-13 spiked in the BN rats after one challenge and then waned with multiple challenges, whereas levels were low in L and SD rats throughout the experimental regime (Fig. 4C).

HDM-specific lymphoproliferative responses. Systemic sensitization to HDM induced significantly higher HDM-specific lymphoproliferative responses in BN rats compared with L rats that received the same treatment (Fig. 5A). SD rats had intermediate responses. Local sensitization to HDM also induced significantly higher responses in BN rats compared with L rats following two or more challenges (Fig. 5B). Responses in SD rats were initially low but developed to the same level as BN rats with successive IT challenges (Fig. 5B).

HDM-specific serum IgG and serum IgE. Among systemically sensitized rats, both BN and SD had significantly higher HDM-specific serum IgG than L rats on postchallenge day 2 (Fig. 6A). Significant increases in

allergen-specific serum IgG levels were observed in the sensitized rats of all strains compared with their controls. HDM-specific IgG titers developed more slowly in the locally immunized rats of each strain but with successive challenges increased above the levels of the systemically immunized animals challenged one time (Fig. 6B).

Systemic sensitization with HDM produced significant increases in HDM-specific serum IgE in all three strains compared with their sham-sensitized controls, and BN and SD rats had significantly higher allergen-specific serum IgE than L rats (Fig. 7A). In general, systemic sensitization produced greater levels of allergen-specific serum IgE in all three strains than local sensitization did. Only BN rats produced significant levels of HDM-specific serum IgE among rats that were sensitized locally, and these levels did not develop until the second challenge but were further boosted after five (Fig. 7B).

Histopathology. Lung histopathology was evaluated two days after challenge in systemically sensitized rats and in locally sensitized rats that received a single IT challenge. Both BN and SD rats that were systemically sensitized to HDM had significantly more lung lesions than L rats with the same treatment (Fig. 8). HDM-sensitized rats of all three strains had greater severity of lung lesions than their respective sham-sensitized controls; however, control levels in BN rats were significantly higher than in L and SD rats. Among locally sensitized rats, BN had significantly more lung lesions than both SD and L, and SD had significantly greater numbers of lesions than L (Fig. 8). Although the number of lesions differed between SD and L, the characteristics of the lesions were identical in these two strains. By contrast, BN rats were distinct from SD and L rats in their histopathology. Unlike sham-sensitized SD and L rats, sham-sensitized BN rats pre-

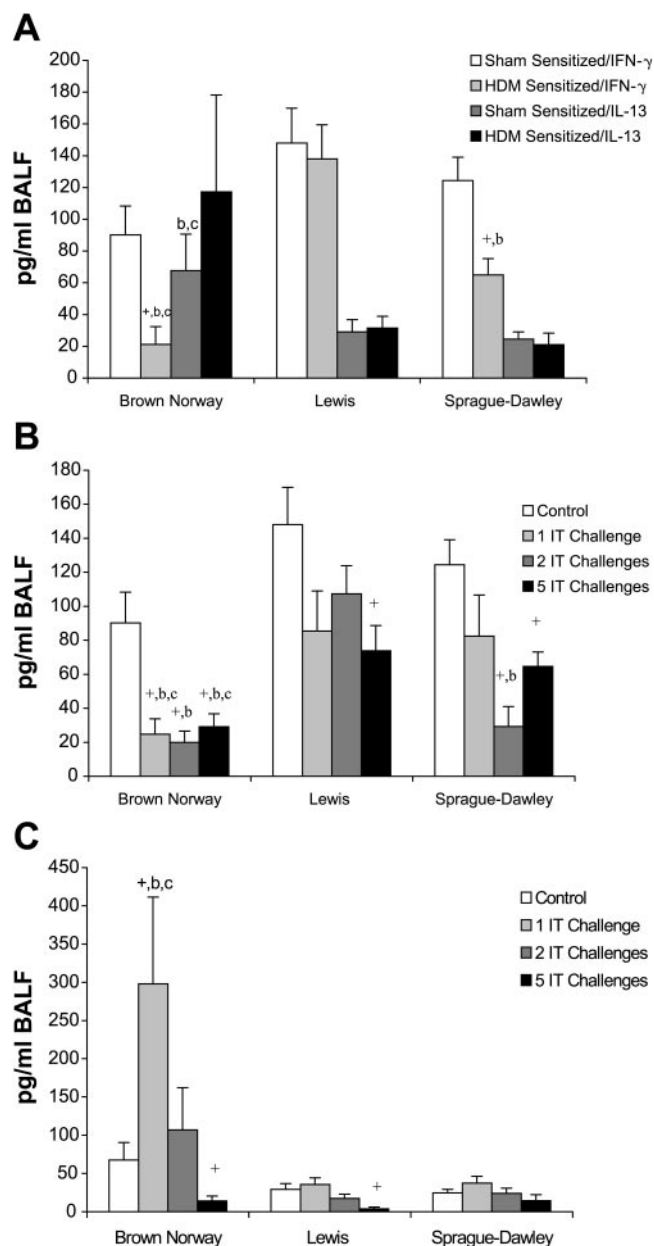


Fig. 4. Th1 and Th2 cytokines in the BALF of rats either sham- or HDM-sensitized by SC and IT routes. IFN- γ (Th1) and IL-13 (Th2) concentrations in systemically (SC) sensitized rats (A), IFN- γ (Th1) concentrations in locally sensitized rats (B), and IL-13 (Th2) concentrations in locally sensitized rats (C) on postchallenge day 2 following IT challenge with HDM. Data are presented as means $\pm$ SE for each treatment group ($n = 4-6$) and were analyzed by 2-sample t -test (with 2-tailed criterion). Significance was determined at $P < 0.05$. ⁺Significantly different from sham-sensitized treatment group within strain; ^bsignificantly different from L rats that received the same treatment; ^csignificantly different from SD rats that received the same treatment.

sented atypical multifocal granulomatous pneumonia, characterized by the presence of eosinophils, lymphocytes, macrophages, and multinucleated giant cells in the alveolar spaces. In BN rats, granulomatous pneumonitis accounted for a significant portion of the lesions, whereas eosinophilic and lymphocytic infiltration around the small blood vessels primarily charac-

terized lesions observed in SD and L rats. In general, the degree of inflammatory lesions was more severe in systemically sensitized rats than in locally sensitized rats in both SD and L, whereas in BN rats, local sensitization produced more severe lesions than systemic sensitization (Fig. 8). Goblet cell hyperplasia and hypertrophy in both locally and systemically sensitized BN rats was more pronounced than in either SD or L rats with the same treatment. Mast cells were not found in significant numbers, regardless of strain.

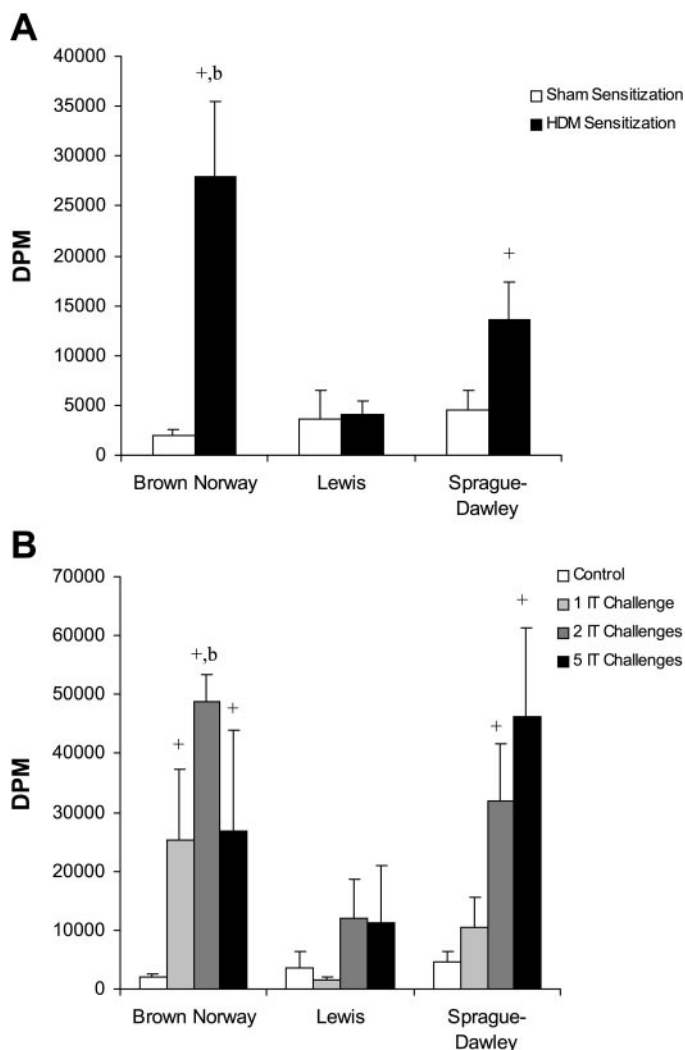


Fig. 5. Lymphocyte proliferation in response to HDM in rats either sham or HDM sensitized by SC and IT routes. Data are expressed as the difference in disintegrations per min (dpm) between a sample cultured with HDM and the same sample culture in media alone. HDM-specific lymphocyte proliferation in systemically (SC, A) and locally (B) sensitized rats on postchallenge day 2, following IT challenge with HDM. Data are presented as means $\pm$ SE for each treatment group ($n = 4-6$) and were analyzed by either 1-way (by strain) or 2-way (by strain and treatment) ANOVA, followed by pairwise comparisons among strains and various strain-treatment combinations. Significance was determined at $P < 0.05$. ⁺Significantly different from sham-sensitized treatment group within strain; ^bsignificantly different from L rats that received the same treatment.

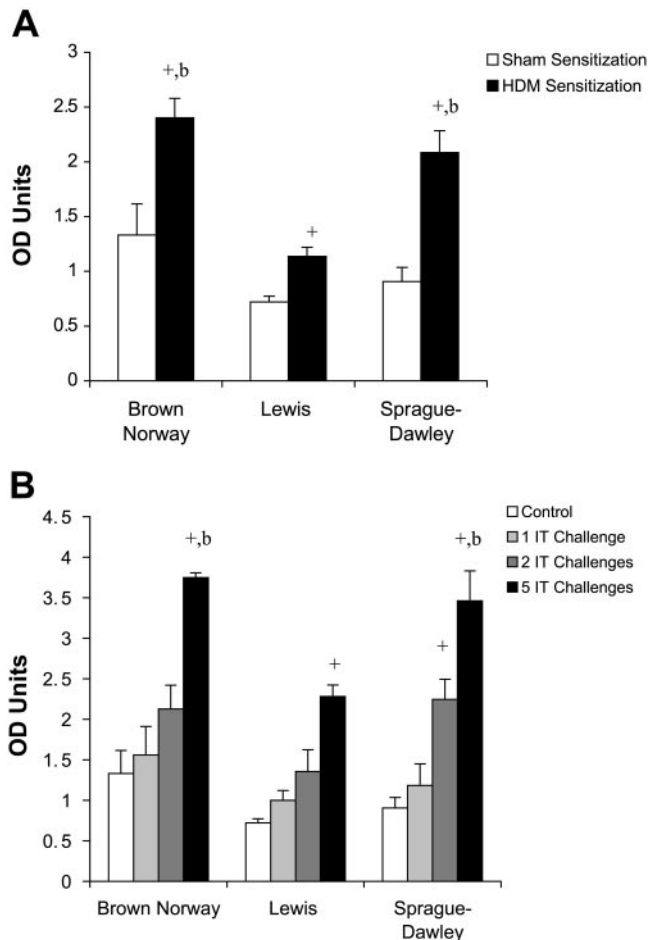


Fig. 6. HDM-specific serum IgG levels derived from whole blood of rats either sham or HDM sensitized by SC and IT routes. HDM-specific serum IgG levels in systemically (SC, A) and locally (B) sensitized rats on postchallenge day 2, following IT challenge with HDM. Data are presented as means $\pm$ SE for each treatment group ($n = 4-6$) and were analyzed by either 1-way (by strain) or 2-way (by strain and treatment) ANOVA, followed by pairwise comparisons among strains and various strain-treatment combinations. OD, optical density. Significance was determined at $P < 0.05$. ⁺Significantly different from sham-sensitized treatment group within strain; ^bsignificantly different from L rats that received the same treatment.

DISCUSSION

The genetic basis of allergic asthma is becoming better understood through animal studies, which show how genotypic variations result in characteristic phenotypic outcomes. It is well supported in experimental rodent literature that susceptibility to allergic sensitization and physiological responsiveness to allergen challenge in the lung are strain dependent (12, 13, 18, 21, 31). The observed differences among both rat and mouse strains in responsiveness to allergens and in their phenotypes of allergic airway disease have provided opportunities to select strains that best simulate allergic asthma and to identify genes that are causally linked to the disease. However, there has been little integration of immune function, airway responses, subsequent pathology, and antioxidant responses in experimentally induced respiratory allergy. In this study we

report striking strain differences in immune responses to allergen, which were associated with Th2 cytokine polarization, increased pulmonary inflammation, and altered antioxidant balance.

One of the hallmarks of allergic asthma is pulmonary eosinophilia, which has been described kinetically in OVA-allergic BN rats (21). Our data showing increased BALF eosinophils in HDM-challenged BN rats agree with earlier studies with OVA, which demonstrated greater responsiveness among BNs in antibody production, eosinophilic inflammation (21), and increased mucus production (19) compared with other rat strains. The minimal pulmonary inflammation and virtually no allergen-specific IgE titers in L rats confirm previous observations by Schneider et al. (21), who reported that two rat strains of a Th1 phenotype demonstrate relatively weak responses to allergen challenge. In this paper we have extended these findings to

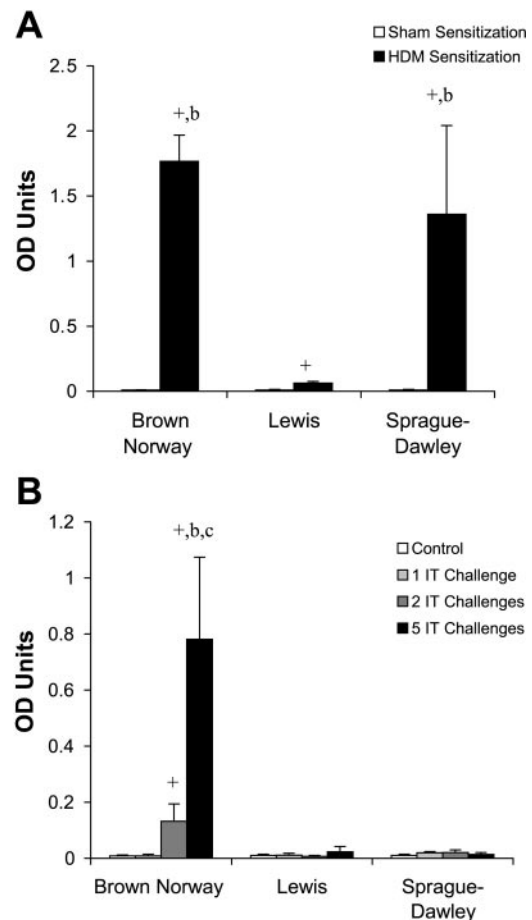


Fig. 7. HDM-specific serum IgE levels derived from whole blood of rats either sham or HDM sensitized by SC and IT routes. HDM-specific serum IgE levels in systemically (SC, A) and locally (B) sensitized rats on postchallenge day 2, following IT challenge with HDM. Data are presented as means $\pm$ SE for each treatment group ($n = 4-6$) and were analyzed by either 1-way (by strain) or 2-way (by strain and treatment) ANOVA, followed by pairwise comparisons among strains and various strain-treatment combinations. Significance was determined at $P < 0.05$. ⁺Significantly different from sham-sensitized treatment group within strain; ^bsignificantly different from L rats that received the same treatment; ^csignificantly different from SD rats that received the same treatment.

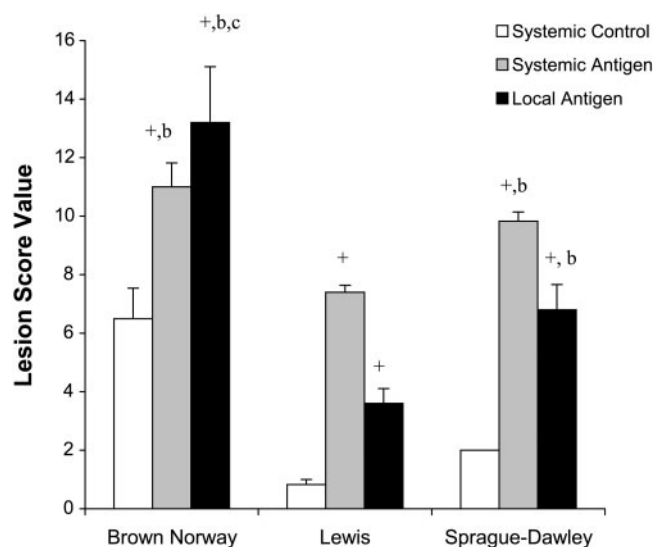


Fig. 8. Strain differences in lung pathology (total lung lesion scores) 2 days after a single IT challenge with HDM. Rats were sham sensitized by SC injection with adjuvant alone or sensitized either systemically (SC) or locally (IT) with HDM then IT challenged with HDM. Data are presented as means $\pm$ SE for each treatment group ($n = 4-6$) and were analyzed by either 1-way (by strain) or 2-way (by strain and treatment) ANOVA, followed by pairwise comparisons among strains and various strain-treatment combinations. Significance was determined at $P < 0.05$. *Significantly different from sham-sensitized treatment group within strain; ^bsignificantly different from L rats that received the same treatment; ^csignificantly different from SD rats that received the same treatment.

include antigen-induced IARs, changes in allergen-specific T cell function, Th1/Th2 cytokine measurements, and antioxidant levels to gain an integrated perspective of strain differences in allergic phenotypes. In addition, the two presumed phenotypic Th1 rat strains (SD and L) could be separated by differences in their IAR and HDM-specific lymphocyte proliferation. Specifically, following multiple allergen challenges, it was found that SD rats became responsive to allergen, whereas L rats did not. SD rats were more like BNs in their levels of HDM-specific IgG and intermediate between BN and L rats with regard to IFN- γ levels in the BALF, suggesting a range of Th1/Th2 allergic phenotypes among rat strains.

It has been previously demonstrated that pulmonary resistance in response to dry-gas hyperpnea challenge is increased in BN rats compared with ACI, L, and Fischer 344 rats and is associated with the release of tachykinins and cysteinyl leukotrienes (32). Although levels of these proteins were not measured in the present study, we have observed significantly elevated levels of cysteinyl leukotrienes in HDM-allergic BN rats exposed to diesel exhaust particles (unpublished observation). Other mediators released from alveolar macrophages, such as nitric oxide, IL-10, and TNF, have also been suggested to explain the differences in allergic susceptibility between SD and BN rats (3).

Earlier studies in rats sensitized and challenged with *Der p 1* (from *Dermatophagoides pteronyssinus*) (26) demonstrated the high IgE responder status of BN compared with Wistar and Lou/M rats and that the low

IgE responder WAG (derivative of the Wistar rat) rat was 1,000 times more sensitive to tolerance induction by repeated aerosol challenges with OVA than BN rats (22). In the present study, increased production of HDM-specific serum IgE and IgG was observed in all three strains with successive IT challenges, and the magnitude of the IgE response reflected the responder status of each strain (BN > SD > L). This trend was also observed in T cell function and the IAR. In contrast, eosinophil recruitment and IL-13 (in BN), IFN- γ (in L and SD), and total protein and GSH concentrations in BALF of all strains decreased over time, indicating altered response kinetics or some form of tolerance building with repeated IT challenge. Together the results show that a sustained cellular and humoral immune response over multiple allergen challenges is not necessarily accompanied by a sustained and progressive release of Th1 or Th2 cytokines, eosinophil recruitment, or tissue damage. A prolonged study, including additional weeks of allergen challenge, would more clearly illustrate strain differences in tolerance induction and immune regulation under a repeated exposure regime.

Hall et al. (6) demonstrated that SD and BN rats had increased serum IgG responses to intrabronchial infection with *Bordetella pertussis* compared with L and Hooded Lister rats. In that study, SD and L rats had the lowest baseline total serum IgE levels and showed the most increase in total serum IgE after infection (6). Although these results show the advantage of low baseline responses in models of infection and immunity, IgE responsiveness among BN, SD, and L rats was demonstrated as high, intermediate, and low, respectively (6), indicating a relative weakness in humoral immune responses among L rats. Rat strain differences in the predominance of humoral vs. cellular immunity have been reported in a model of autoimmune disease induced by immunization with human myeloperoxidase (2). In that model, BN rats have stronger humoral immune responses (antibody responses), whereas L rats have more potent cellular immune responses, as demonstrated by (Th1-mediated) delayed type hypersensitivity responses to myeloperoxidase (2). The same strain-specific tendencies toward humoral and cellular immunity have been shown in the present model of allergic airways disease.

L and SD rats had lower baseline responses (in nonsensitized rats) than BN rats with respect to histopathology lesions, IL-13 levels, and inflammatory cells (especially eosinophils) in the BALF. On the other hand, L and SD rats had higher baseline levels of BALF IFN- γ , and SD rats had higher baseline BALF GSH levels. These differences in Th1 cytokine levels and antioxidant reserves in the BALF of nonsensitized rats may have accounted for the observed differences in responsiveness to allergen and lung injury among sensitized rats. Oral administration of the antioxidant taurine has been shown to significantly reduce eosinophil influx as well as vascular leakage into the BALF of BN rats after allergen challenge (4). Shvedova et al. (23) demonstrated that OVA challenge in sensitized

guinea pigs (Hartley strain) induced a significant increase in eosinophils and lipid peroxidation products with a coincident decrease in AA and α -tocopherol concentrations in BALF, suggesting that allergen challenge induces an oxidative stress response in the lung. The present paper is the first report indicating strain-specific differences in endogenous antioxidant levels in an allergic rat model. Peak antioxidant levels were generally lower in BN rats than in L and SD rats, among both allergen-sensitized and nonsensitized groups. These reduced antioxidant levels may be indicative of a difference in the ability of BN rats to adapt to oxidative stress induced by allergen challenge. It is possible that the differences in endogenous antioxidant levels may play a role in the initiation of immune responses and subsequent hypersensitivity disease. Further investigation of the development of nonatopic and atopic immune responses to allergens in different rat strains (and F1 hybrids between responder and nonresponder strains) will help identify individual genes that confer susceptibility to allergic airway disease.

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