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Comparison of allergic lung disease in three mouse strains after systemic or mucosal sensitization with ovalbumin antigen

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Abstract Murine models of allergic lung disease have many similar traits to asthma in humans and can be used to investigate mechanisms of allergic sensitization and susceptibility factors associated with disease severity. The purpose of this study was to determine strain differences in allergic airway inflammation, immunoglobulin production, and changes in respiratory responses between systemic and mucosal sensitization routes in BALB/cJ, FVB/NJ, and C57BL/6J, and to provide correlations between immune and pathophysiological endpoints. After a single intranasal ovalbumin (OVA) challenge, all three strains of mice systemically sensitized with OVA and adjuvant exhibited higher airflow limitation than non-sensitized mice. No changes were seen in mice that were pre-sensitized via the nose with OVA. Systemic sensitization resulted in an elevated response to methacholine (MCH) in BALB/cJ and FVB/NJ mice and elevated total and OVA-specific IgE levels and pulmonary eosinophils in all three strains. The mucosal sensitization and challenge produced weaker responses in the same general pattern with the C57BL/6J strain producing less serum IgE, IL5, IL13, and eosinophils in lung fluid than the other two strains. The converse was found for IL6 where the C57BL/6J mice had more than twice the amount of this cytokine. The results show that the FVB/NJ and BALB/cJ mice are higher Th2-responders than the C57BL/6J mice and that the levels of pulmonary eosinophilia and cytokines did not fully track with MCH responsiveness. These differences illustrate the need to assess multiple endpoints to provide clearer associations between immune responses and type and severity of allergic lung disease.

Keywords Asthma · Genetic variability · Pulmonary function · Th2-type response

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Introduction

Asthma has emerged as a major health concern since the mid 1970s. During the period of 1980–1994, the reported incidence of asthma rose 75% in the United States (Forecasted State-Specific Estimates of Self-Reported Asthma Prevalence—United States 1998). By 1998, seventeen million Americans (4.8 million of whom were children) were diagnosed with asthma. An estimated 5,000 asthma-related deaths occur annually in the US (Focus On Asthma, National Institute of Allergy and Infectious Disease (NIAID)). The development of asthma is associated with a complex combination of genetic, immune, environmental, and socio-economic factors. Studies in humans are usually restricted to lung-function testing, clinical history, and skin testing for allergens, with subsequent in vitro assessment of immune function. Animal models that mimic the pulmonary features observed in human asthma allow for more invasive functional assessment in vivo and offer important tools to study the genetic factors underlying susceptibility as well as mechanisms of disease induction (Wills-Karp

2000; Leong and Huston 2001; Kumar and Foster 2002; Gelfand 2002).

After systemic immunization with a Th2-stimulating adjuvant such as aluminum hydroxide ($Al(OH)_3$) and a subsequent pulmonary challenge, various strains of mice develop airway hyper-responsiveness which has been regarded as an outcome indicator of asthma (Wanner et al. 1990). Several studies have shown broad genetic variation in reactivity with this immunization regime which results in differential severity of disease (Brewer et al. 1999; Ichinose et al. 1997; Wills-Karp and Ewart 1997; Whitehead et al. 2003). In addition to genetic differences in various mouse strains, different antigen sensitization and challenge protocols also result in varying magnitudes of disease severity. Like asthma in humans, multiple pathways in mice can lead to the same clinical phenotype. For example, airway hyperresponsiveness (AHR) can be induced by numerous cytokine pathways and by eosinophil-dependent and -independent mechanisms (Corry et al. 1996; Foster et al. 1996; Lack et al. 1995; Coyle et al. 1996; Mehlhop et al. 1997). To illustrate this, Foster et al. (1996) induced AHR in wild type C57BL/6J by intraperitoneal injection of OVA followed by repeated OVA aerosol exposures and failed to produce this effect in IL-5 knockout animals. Corry et al. (1996) showed that sensitized and challenged BALB/cJ mice exhibited increased levels of IL-4, IgE, and AHR, and while these effects were prevented by treatment with an anti-IL-4 mAb, anti-IL-5 mAb inhibited airway eosinophilia but not AHR. These studies, together, indicate that AHR can be independent of IL-5 and eosinophils.

Because of these ambiguous findings, it is important to consider and measure multiple biological endpoints in order to fully understand the regulatory pathways, cytokines, and immune endpoints that are associated with allergic lung disease. In addition, many of the allergy models use systemic sensitization with adjuvant which bypasses mucosal processing and local sensitization and may provide less realistic information than mucosally administered antigen. In general, naïve rodents exposed to inhaled proteins develop immune tolerance to avoid producing immune-mediated disease in the lung (de Heer et al. 2004; Ostroukhova et al. 2004). However, under some circumstances, the presence of low levels of LPS may induce Th2 aeroallergen sensitization and the development of allergic responses (Eisenbarth et al. 2002). Mild local airway allergic responses induced by intranasal administration of OVA have been used to test allergic effects induced by air pollution components (Steerenberg et al. 2003). In this study, we sensitized three mouse strains by systemic and local sensitization routes and compared development of immunity and pulmonary allergic reactions. The association of immune parameters with phenotypic indicators of disease under contrasting sensitization regimes provides additional information to select biomarkers that are directly associated with subsequent expression of disease following antigen challenge.

Materials and methods

Animals Female mice from three strains (BALB/cJ, FVB/NJ and C57BL/6J) were purchased from Jackson Laboratories (Bar Harbor, ME) and used at 8–10 weeks of age. Mice were housed in American Association for Accreditation of Laboratory Animal Care (AAALAC)-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. The mice were housed in plastic shoebox-type cages suspended over absorbent bedding and were maintained on a 12-h diurnal cycle. Food and water were provided ad libitum. Sentinel animals were selected randomly and serologically tested upon arrival, and throughout the study, were free of Sendai virus, pneumonia virus, and a variety of other rodent viruses, and Mycoplasma sp.

Experimental design Mice from each strain were divided into four groups: systemic sensitization, sham systemic sensitization, local sensitization, and sham local sensitization. Systemically sensitized mice were intraperitoneally (i.p.) injected twice at an interval of 2 weeks with a suspension containing 20 μg of OVA (grade V, Sigma Chemical Co., St. Louis, MO) and 2 mg of $Al(OH)_3$ (Sigma Chemical Co., St. Louis, MO) in 0.5 ml of saline. Sham-sensitized mice were injected with $Al(OH)_3$ alone. Locally sensitized mice were administered with 10 μg of OVA in 25 μl saline through each nostril at day 1 and 14. Sham-sensitized mice were intranasally (IN) exposed to saline alone. Twelve days after the last sensitization, all the mice were IN-challenged three times over a 6-day period with 20 μg of OVA. Immediate responses to antigen were measured in a whole-body plethysmograph after each challenge. Two days after the final challenge, pulmonary responses to inhaled MCH were measured. After this, the mice were rested for 1 h prior to euthanasia, bronchoalveolar lavage (BAL), and isolation of lung tissue and serum samples.

Airflow limitation immediately following specific antigen challenges

Airflow limitation was measured immediately after each exposure to OVA in unrestrained mice using whole-body plethysmography (Buxco Electronics, Troy, NY). The resulting calculated parameter enhanced pause (PenH) is a

unitless parameter which strongly correlates with lung resistance (Hoffmann et al. 1997). Baseline PenH measurements for each animal were recorded for 10 min and averaged. Animals were then intranasal-exposed to OVA, and placed back in the chambers within 5 min of dosing. PenH readings were then monitored and averaged over a 1-h post-challenge period.

Airflow limitation following MCH challenge Two days after the last OVA challenge, respiratory responses to increasing concentrations of aerosolized MCH were measured in unanesthetized, unrestrained mice in a 12-chamber Buxco system. PenH was used as an index of ventilatory timing as previously described (Gavett et al. 2003; Hamelmann et al. 1997). After measuring baseline parameters for 10 min, an aerosol of saline or MCH in increasing concentrations (6.25, 12.5, and 25 mg/ml) was nebulized through an inlet of the chamber. Airflow was 6 L/min. The response to saline or MCH was measured over the aerosolization period (1 min), an aerosol-drying step (2 min), and an additional 4-min period. Changes in breathing patterns were detected by a pressure transducer and quantified.

Pulmonary lavage cytology Bronchoalveolar lavage fluid (BALF) samples were collected as previously described (Gilmour et al. 2004). Briefly, mice were euthanized with 0.2 ml of a 5:1 pentobarbital solution (50 mg/kg of pentobarbital sodium i.p.). The left mainstem bronchus was isolated, clamped, and the trachea cannulated. The right lung lobes were lavaged three times with warm Hanks balanced salt solution (HBSS, body weight×35 mL/kg 37°C). BAL was centrifuged at 800×g for 10 min and the supernates were stored for biochemistry (4°C) and cytokine assessment (−80°C). Lavage fluid was analyzed for biochemical markers of lung injury and edema (LDH, protein, albumin, NAG). Cells were resuspended in 1,000 μl minimal essential medium (MEM) containing 2.5% fetal bovine serum (FBS), and total BALF cell counts were obtained by Coulter counter (Coulter Corp., Miami, FL). Additionally, 200 μl of resuspended cells were adhered in duplicate onto glass slides using a Cytospin (Shandon, Pittsburgh, PA) and subsequently stained with Diff Quik solution (American Scientific, McGraw Park, PA) for cell differentiation determination, with at least 200 cells counted from each slide.

BALF biochemical analysis Total protein, microalbumin (MIA), N-acetyl glucosaminidase (NAG) and lactate dehydrogenase (LDH) assays were performed using a KONELAB 30 clinical chemistry spectrophotometer analyzer (Thermo Clinical Labsystems, Espoo, Finland) as described previously (Singh et al. 2004).

BALF cytokine analysis Concentrations of IL-1, 2, 4, 5, 6, 10, 12, 13, IFN-γ, GM-CSF, and TNF-α in whole lung lavage fluid were determined using commercial multiplexed fluorescent bead-based immunoassays (Upstate LINCO Research, St. Charles, MO, USA). Briefly, standard concentrations of the respective cytokines (50 μl) were placed in duplicate into the wells of a 96-well filtration plate. Standards were one-half dilutions, ranging from 3.9 to 250 pg/ml of recombinant mouse cytokines. Unknown samples consisting of 25 μl of whole lung lavage fluid were added to the additional wells. The samples were incubated with 25 μl of anti-mouse multicytokine beads, specific for mouse cytokines, at 4°C for 18 h on a plate shaker. Unbound cytokine beads were filtered through the wells using a vacuum manifold. Biotin anti-mouse multicytokine reporter was added to each well as a secondary/detection antibody and incubated at 37°C in the dark on a plate shaker for 2 h. Streptavidin–phycoerythrin (25 μl) was added to the wells and incubated at 37°C in the dark on a plate shaker for 2 h. The addition of 25 μl of stop solution terminated the reactions. The samples were read using a Luminex^{100} Instrument (Luminex, Austin, TX) in which a minimum of 50 beads per cytokine for each sample were analyzed. Blank values were subtracted from all readings. The lower limits of sensitivity for cytokines ranged from 0.3 to 10.5 pg/ml, respectively.

OVA-specific IgE, IgG1, IgG2a, and total IgE Microtiter plates (Costar Corp., Cambridge, MA) were coated with OVA (10 μg/ml) in phosphate-buffered saline (PBS), pH 7.3, and incubated overnight at 4°C. Following blocking with buffer (PBS 0.05% Tween 20 plus 2% BSA), mouse IgE, IgG1, IgG2a standard, and serum samples were added to the plates and incubated overnight at 4°C. The next day, plates were treated successively with biotinylated detection antibody (rat anti-mouse IgE, IgG1, IgG2a; Pharmingen) and horseradish peroxidase–streptavidin (1:8,000), washed, and incubated for 1 h at room temperature between each of these steps. Finally, TMB substrate (Dako Corp.) was applied and, 30 min later, 1 M H_3PO_4 was added to stop the reaction. Optical density was read on a Thermomax® Plate Reader (Molecular Devices Corp., Menlo Park, CA) at a wavelength of 450 nm. Softmax Pro® version 2.6.1 (Molecular Devices Corp.) was used to calculate and convert from optical density to protein concentrations. The limit of detection for the assay was 6.25 (BALF) and 2.5 ng/ml (serum).

Histopathology Lungs were fixed in 10% buffered formalin acetate (Fisher Scientific, Fair Lawn, NJ) and, after 24 h, washed in water and resuspended with 70% ethanol and stored at 4°C until processed. Lungs were embedded in paraffin, sectioned along the mainstem bronchus and

stained with hematoxylin–eosin solution prior to pathological examination by a certified veterinary pathologist (Experimental Pathology Laboratories, INC. RTP, NC). The degree of severity of inflammatory, degenerative, and proliferate changes was graded on a scale of 1–5 (1: minimal; 2: slight/mild; 3: moderate; 4: moderately severe; 5: severe/high). The pathology scores for lung sections were summarized as follows: incidence is the number of mice exhibiting certain pathology; severity is the numeric average of the pathology scores for a particular lesion per treatment group.

Statistical analyses Student's t test was used to determine differences from control values. Values with $p \leq 0.05$ were considered significant. Statistical analyses were performed by GraphPad Prism 4.03 (GraphPad Software, Inc., San Diego, CA). The Pearson correlation coefficient amongst different endpoints was calculated by SAS 2.0 (SAS Institute Inc., Cary, NC).

Results

Airflow limitation immediately following multiple antigen challenges Immediate respiratory responses were measured for 1 h following each intranasal OVA exposure (Fig. 1). In most cases, antigen challenge on its own caused a change in response from baseline. All three strains of mice systemically sensitized with OVA and $Al(OH)_3$ exhibited higher immediate airflow limitation than non-sensitized mice ($Al(OH)_3$ alone) after a single intranasal challenge (C1). Each strain, however, showed a distinct pattern of response following multiple antigen challenges depending upon the sensitization history. Systemically immunized BALB/cJ mice had lower responses to the third antigen challenge compared to sham-sensitized controls. C57BL/6J mice showed significant differences from controls after three challenges (C3), while FVB/NJ mice had strong immediate responses after one (C1) and two challenges (C2) but no effect following the third exposure. Assessment of immediate responses in mice locally sensitized and then challenged with antigen showed even less clear immune-mediated effects (data not shown). Overall, only the C57BL/6J mice challenged three times had significant increases in responses compared to sham-sensitized animals.

Airflow limitation following MCH challenge Only systemic sensitization BALB/cJ mice showed a significant increased airflow limitation to MCH compared to alum-only injected controls (Fig. 2a). There was also a trend of higher reactivity in this strain after local sensitization; however, this was not statistically significant (data not shown; $p = 0.08$). C57BL/6J mice did not show differences between sensitized and control groups regardless of immunization route (Fig. 2b). The FVB/NJ responses were more complex in that systemic immunization resulted in strong responses to MCH at the intermediate aerosol concentrations but no difference from controls at the high MCH level (Fig. 2c). In contrast, local sensitization resulted in lower responses than sham-sensitized animals which were then challenged (data not shown).

BALF biochemical analysis Under systemic sensitization conditions, all three strains demonstrated significant increases in total protein (Fig. 3a), MIA, and NAG (data not shown) after challenge in the BALF compared with

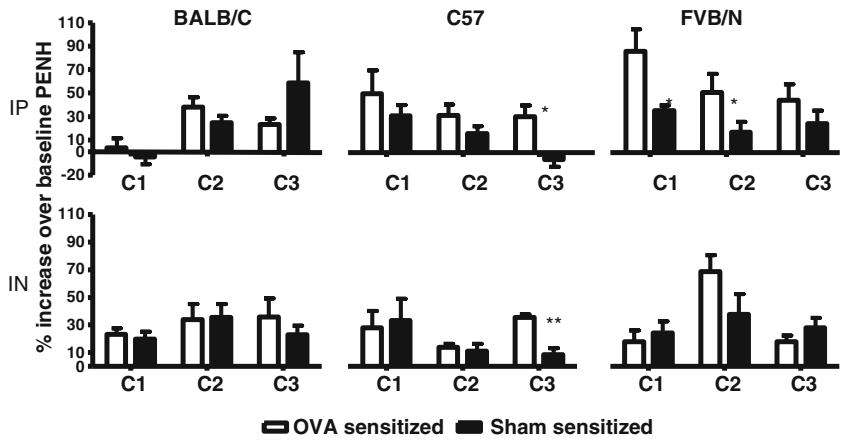

Fig. 1 Immediate respiratory physiological responses in three strains of mice after IN challenge 1 (C1), challenge 2 (C2), challenge 3 (C3) with OVA at different time points (n=5). Upper panel systemic sensitized with OVA or alum-only injected controls, lower panel local sensitized with OVA or saline controls. Data are shown as mean±S.E. from each group. Open bars represent the increased percentage of PENH over baseline from OVA-sensitized mice. Solid bars represent the increased percentage of PENH over baseline from sham-sensitized control mice. Asterisks significant difference from saline-sensitized control mice. *$0.01<p<0.05$; **$0.001<p<0.01$

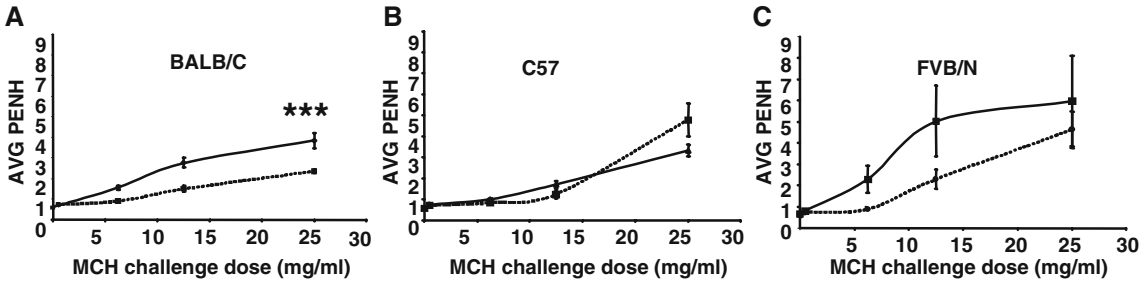

Fig. 2 Dose–response curve of respiratory resistance after aerosolized methacholine (MCH) exposure in three strains of mice systemically sensitized and then intranasally challenged with OVA (solid line) or saline as control (dot line). Data are shown as mean±S.E. from each group (n=5). ***p<0.001. Statistical analyses were performed by two-way ANOVA

alum-only injected controls. Only systemically sensitized BALB/cJ had significantly greater amounts of LDH compared to sham-sensitized controls. There were no significant differences in levels of LDH or MIA in BALF in locally sensitized mice of any strain compared with their respective sham-sensitized controls (Fig. 3).

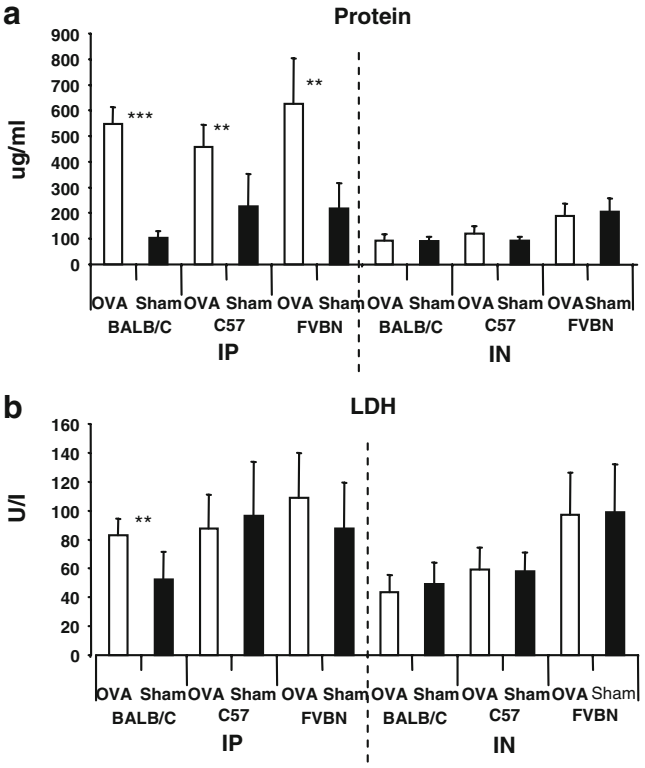

Fig. 3 Total protein (a) and LDH (b) level in BALF of three strains of mice either saline- or OVA-sensitized and challenged with OVA. Total protein concentrations in systemically (IP) and locally (IN) sensitized mice on post-challenge day 2 following IN challenge with OVA. Data are presented as means±standard deviation for each treatment group (n=5) and were analyzed by Student's t test. Significance was determined at p<0.05. Asterisks significantly different from sham-sensitized treatment group within strain. **0.001<p<0.01; ***p<0.001

BALF cell differentials Among both systemically and locally sensitized mice, there were significant increases in total cell numbers in BALF over sham-sensitized mice which were primarily due to eosinophils (Fig. 4). For each strain, the absolute number and percentages of eosinophils were higher in the systemically immunized animals compared to mucosally sensitized animals. The three mouse strains did not show appreciable differences in percent eosinophils for any active immunization indicating that mucosal sensitization could still result in appreciable eosinophilia post-challenge. There were no differences in percentages of macrophages, polymorphonuclear cells (PMNs)/neutrophils, or lymphocytes in the BALF (data not shown).

Serum IgE and IgG1 Systemic OVA sensitization caused a significant increase in OVA-specific serum IgE (Fig. 5) and IgG_1 (data not shown) levels in BALB/cJ and C57BL/6J mice. Interestingly, sham-sensitized FVB/NJ mice generated the same levels of OVA-specific IgE and IgG_1 as OVA-

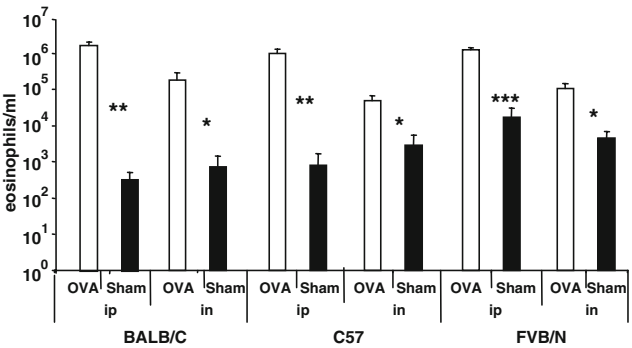

Fig. 4 Total eosinophil cell in bronchoalveolar lavage fluid (BALF) from three strains of mice either saline- or OVA-sensitized and challenged with OVA. Total eosinophil cells per milliliter BALF in systemically (IP) or locally (IN) sensitized mice on post-challenge day 2, following IN challenge with OVA. Data are presented as means± standard deviation for each treatment group (n=5) and were analyzed by Student's t test. Significance was determined at p<0.05. Asterisks significantly different from sham-sensitized treatment group within strain. *0.01<p<0.05; **0.001<p<0.01; ***p<0.001

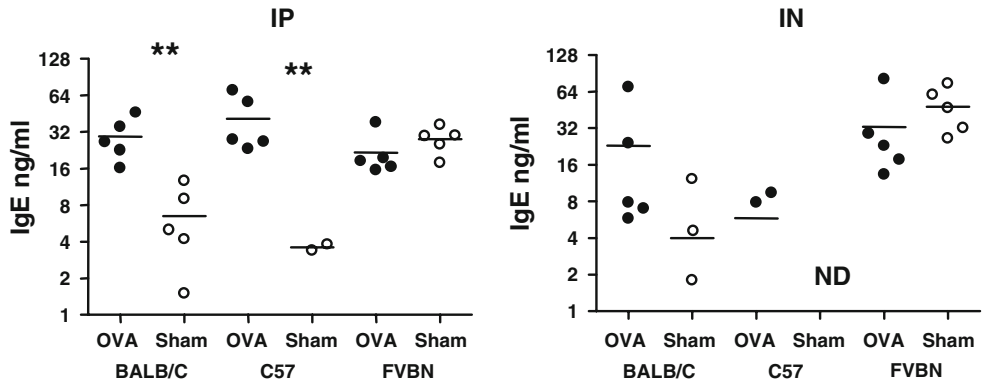

Fig. 5 OVA-specific serum IgE levels from three strains of mice either saline- or OVA-sensitized and challenged with OVA. OVA-specific serum IgE levels in systemically (IP) and locally (IN) sensitized mice on post-challenge day 2, following IN challenge with OVA. Each data point represents a mouse. Data are presented as means±standard deviation for each treatment group (n=5) and were analyzed by Student's t test. Significance was determined at $p<0.05$. *Significantly different from sham-sensitized treatment group within strain. **$0.001<p<0.01$. ND not detectable

sensitized mice. For the local route of administration, BALB/cJ mice developed strong responses while FVB/NJ again had high levels in both sensitized and control animals. Most C57BL/6J mice did not generate significant OVA-specific serum IgE and IgG1 levels following intranasal sensitization and challenge.

Cytokines OVA systemic sensitization resulted in increased levels of IL-4, 5, 6, and TNF-α compared to alum-only injected controls in all three strains (Table 1). Only BALB/cJ and FVB/NJ mice produced significantly higher IL-13. OVA local sensitization resulted in lower cytokine responses which did not reach significance over control animals because of substantial variability in treatment and control samples. All other cytokines (IL-1, 2, 10, 12, IFN-γ, GM-CSF) were not detectable in any of the treatment groups of strains.

Pathology The lung pathology results correlated well with the bronchoalveolar lavage data. In systemically sensitized mice, there was greater severity of inflammation in each strain compared to the corresponding saline control groups.

Table 1 Cytokines (pg/ml) in the BALF of mice either saline- or OVA-sensitized, and challenged with OVA

Strains	Sensitization		IL4	IL5	IL6	IL13	TNF-α
BALB/cJ	IP	OVA	5.1±1.3	106.9±24.7	27.3±15.4	31.2±9.3	1.8±0.6
		SAL	0.1±0.1	6.8±2.9	3.8±1.3	<3.2	0.5±0.2
		Stat. sig.	–**	–**	–**	–**	–**
C57BL/6J		OVA	9.1±5.4	45.4±39.5	314.1±273.8	17.7±21	1.5±1.3
		SAL	0.4±0.4	14.8±14.3	7.7±6.6	<3.2	0.7±0.5
		Stat. sig.	–**	–**	–**	NS	NS
FVB/NJ		OVA	4.4±1.3	148.7±62.8	149.9±134.8	26.7±16.7	4.0±1.8
		SAL	0.8±1.1	34.5±40.7	12.5±11.8	7.9±3.1	0.6±0.1
		Stat. sig.	–**	–*	–**	–**	NS
BALB/cJ	IN	OVA	1.1±1	26.6±15.8	7.5±6.4	<3.2	0.6±0.4
		SAL	0.0±0.1	3.0±1.2	2.9±1.7	ND	0.6±0.3
		Stat. sig.	–*	NS	NS	NS	NS
C57BL/6J		OVA	0.5±0.6	6.5±0.4	8.7±7.9	ND	0.3±0.2
		SAL	0.1±0.1	6.8±9	4.0±6.1	ND	0.3±0.1
		Stat. sig.	NS	NS	NS	NS	NS
FVB/NJ		OVA	0.6±0.3	20.2±10.7	12.8±5.8	8.5±6.2	0.7±0.4
		SAL	0.3±0.2	13.7±3.9	13.9±10.2	ND	0.3±0.2
		Stat. sig.	NS	NS	NS	NS	NS

Cytokines concentrations in systemically sensitized mice (IP) or in locally sensitized mice (IN) on post-challenge day 2 following IN challenge with OVA. Data are presented as means±standard deviation for each treatment group (n=5) and were analyzed by a two-sample t test (with two-tailed criterion). Asterisks significant difference from saline-sensitized control mice. *$0.01<p<0.05$; **$0.001<p<0.01$; ***$p<0.001$
ND Not detectable, NS not significant

The most severe lesions were seen in the BALB/cJ mice sensitized with OVA. Moderate to moderately severe inflammation (scale grade; 3–4) was seen in the BALB/cJ mice sensitized with OVA. Mild to moderate inflammation (scale grade, 2–3) was seen in the C57BL/6J and FVB/NJ mice sensitized with OVA. No inflammation was seen in the corresponding saline controls. In the locally sensitized mice, there was again a higher severity of inflammation in the BALB/cJ mice when compared to the corresponding saline controls which had minimal changes. In the C57BL/6J and FVB/NJ strains, there were no differences in the severity of lesions compared to the corresponding saline control mice.

Correlation between immune responses and pulmonary airway reactions Different sensitization methods resulted in contrasting immune response patterns in the three mouse strains. Under systemic sensitization conditions, there were no clear correlations among the different immune and pathophysiological endpoints. After local sensitization, BALB/cJ and C57BL/6J mice showed a strong correlation (Pearson correlation coefficient, BALB/Cj R^2=0.9611; C57BL/6J R^2=0.9694) between serum-specific IgE levels and the severity of infiltration of eosinophils in lung which were not correlated to the airway responses to MCH. In contrast, in FVB/NJ mice, the serum-specific IgE level was the only endpoint that correlated to the airway responses to MCH (Pearson correlation coefficient, R^2=0.9556).

Discussion

Animal models have been used for decades to unravel the immunological mechanisms and susceptibility factors associated with allergic lung disease and asthma (Zosky and Sly 2007), and have also been utilized to investigate the efficacy of therapeutics and the adverse effects of inhaled pollutants. Depending on the species and strain of animal, immunization route and frequency, and the antigen type and dose, the resulting phenotype can display a complex mix of cytokines and chemokines, varying levels and type immunity, and a broad range of pathophysiological alterations associated with allergic lung disease and asthma. Because of the many variables associated with these systems, each experimental model should be well-defined in order to link the appropriate route of sensitization, immune function endpoints and cytokine profile with accepted markers of disease such as eosinophils and lung-function changes.

The purpose of this study was to examine strain specific differences in immune and pathological responses to OVA antigen, and to determine the relationship with pulmonary function changes following multiple antigen challenges. We also sought to determine the type and extent of disease in locally sensitized mice to understand differences that may arise from the development of mucosal immunity at the lung surface. In most murine models of allergic lung disease, mice are sensitized once or twice by intraperitoneal injection of OVA with adjuvant followed by three airway allergen challenges (Hopfenspirger and Agrawal 2002; Gerhold et al. 2002; Brewer et al. 1999; Ichinose et al. 1997; Wills-Karp and Ewart 1997; Whitehead et al. 2003) and, to a greater or lesser extent, results in the development of atopy (IgE antibody), increased lung injury, pulmonary eosinophilia, and increased responsiveness to MCH. Our results confirmed this generalized phenotype but also illustrated strain differences which may clarify why not all studies report the same effect. For example the three different strains produced quite different cytokine profiles and lung-function changes despite having near-equivalent levels of pulmonary eosinophils, suggesting that these endpoints are not necessarily linked. In addition, some biomarkers including IgE antibody and eosinophils were also significantly elevated in locally sensitized animals despite much lower cytokine levels, and no markers of lung injury or altered pulmonary function. From this, we conclude that the mucosal sensitization paradigm can be used to study humoral and inflammatory aspects of immune-mediated lung disease, but the initial systemic immunization is needed to induce other disease endpoints such as methacholine responsiveness and acute lung injury. Our data are, in general, in agreement with those of Whitehead et al. (2003) who reported that of the three strains studied here, the BALB/cJ mouse was the most consistent responder compared to the C57BL/6J and FVB/NJ strain, and also showed that there was significant disparity between AHR responses, Th2 cytokine output and IgE antibody levels amongst the various strains. In addition, however we also assessed responsiveness following local immunization and while the same general hierarchy emerged, it was clear that many of the endpoints could also be elicited in BALB/cJ mice without the use of systemic immunization with adjuvant.

The results also showed that under some circumstances (notably in the C57BL/6J and FVB/NJ mice) pulmonary function changes could be observed immediately after antigen although these changes were not related to any of the conventional immune markers of type I hypersensitivity such as IgE or IL-4. Distinguished from the other two strains, the C57BL/6J strain showed relatively lower levels of IL5 and IL13 but higher IL4 and IL6 production. Despite the lack of IL-5, the C57BL/6J mice had increased eosinophilic infiltration in the lungs suggesting that other chemokines such as eotaxin could have been involved in this effect. The C57BL/6J mice did not display any observable AHR to MCH challenge which has been attributed to IL-13 production (Wills-Karp et al. 1998).

The FVB/NJ is an inbred strain that carries the $Fv1^b$ allele that is sensitive to histamine challenge and is also reported to develop strong airway responsiveness and significant generation of antigen-specific IgE (Taketo et al. 1991). In the present study, both OVA- and sham-sensitized FVB/NJ mice generated high levels of OVA-specific IgE after three local antigen challenges suggesting that these exposures over a 6-day period were sufficient to generate high antibody responses which, in other strains, required an initial sensitization 2 weeks prior to challenge. It would appear that this strain is extremely sensitive and a high responder to antigen, and additional studies with a more limited challenge regime would be required to optimize this model to avoid saturation of immunity in the non-sensitized control animals.

Among the three strains, the BALB/cJ mice presented the best separation of reactivity from controls under both the systemic and local sensitization protocols. This was particularly apparent for OVA-specific IgE and IgG1 antibody, increased Th2-type cytokine secretion, and, in the systemically immunized groups, increased lung protein (as a measure of edema), and responsiveness to MCH. The results also showed that BAL IL-5 was associated with specific serum IgG1 antibody levels under both systemic and local sensitization protocols.

We conclude that the three different stains tested in this study exhibited contrasting pulmonary reaction patterns under the same immunization strategy, suggesting that the kinetics and/or type of immunological pathways differ according to genotype. Importantly, the mucosal sensitization resulted in appreciable antibody and eosinophil responses suggesting that this more realistic route of antigen administration in the absence of adjuvant can be used to study these features of allergic lung disease. The results also indicated, however, that the various markers of allergic lung disease did not necessarily track with each other (e.g., eosinophils and responsiveness to MCH), providing evidence that these endpoints are independent in both their generation and causal relationship with each other. While some studies have found good correlation between these two disease markers in human asthma, other reports have found no association (Kobayashi et al. 2003; O'Byrne et al. 2001). From this study and others, it would appear that many of the accepted phenotypic markers such as IgE, AHR and eosinophils can operate either in tandem or independently to produce allergic lung disease and asthma. Here, we report that three immunologically distinct mouse strains can be manipulated to produce some or all of these endpoints depending upon the immunization regime, and that the BALB/cJ mouse remains the most reliable strain for most significantly expressing the majority of these elements under both local and systemic sensitization protocols.

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