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Rapid report

Tolerance induced by chronic inhaled antigen in a murine asthma model is not mediated by endotoxin

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Abstract

Ovalbumin (OVA)-sensitized wildtype (WT) and endotoxin-resistant (ER) mice developed similar degrees of airways eosinophilia and serum OVA-specific IgE levels after acute aerosolized OVA challenge. WT mice demonstrated methacholine hyperreactivity, whereas ER mice showed no change in responsiveness. With chronic aerosolized OVA challenge, both WT and ER mice developed local tolerance, with resolution of airway eosinophilia but persistence of anti-OVA IgE in serum. Thus, the development of local tolerance with chronic aerosol exposure to OVA is independent of any potential effects of endotoxin in the OVA aerosol solution.

Keywords: Asthma; Endotoxin; Lipopolysaccharide; Mouse; Sensitization; Tolerance

Endotoxin is a potent modulator of the asthma response. Epidemiological studies have implicated endotoxin as a protective factor against the development of asthma [1,2]. Conversely, exposure to endotoxin can lead to exacerbations of existing asthma [3,4]. Similarly, studies in animal asthma models using ovalbumin (OVA) as the inciting allergen have reported both inhibitory and pro-inflammatory effects of endotoxin [5–9]. At least some of the variability of these responses can be attributed to differences in timing and dosing of the endotoxin. Moreover, commercial OVA preparations are highly contaminated with endotoxin. The high-grade chicken egg white albumin (Sigma Grade V OVA, over 98% pure) used in our experiments contains up to 0.4 μg endotoxin per 1 mg OVA (i.e., 0.04% contamination) [10]. Other commercial OVA preparations may contain as high as 10 μg of endotoxin per 1 mg OVA (1% contamination) [5].

Investigations of endotoxin as a modulatory factor in animal models of asthma have focused on the acute aspects of the disease. We have reported a model wherein mice sensitized to OVA develop a biphasic response to inhaled OVA challenge [10]. Acute (3–10 days) aerosol exposure results in Th2 cytokine production, OVA-specific IgE generation, airway eosinophilia, and hyperresponsiveness to methacholine — all characteristics of allergic airway disease (AAD). In contrast, chronic (6–11 week) aerosol exposure results in local inhalational tolerance (LIT), wherein airway inflammatory responses are attenuated but systemic IgE production persists. We attributed the development of LIT to an antigen-dependent mechanism; however, the demonstration that our aerosolized OVA solution contained 0.6–4.1 μg/ml of LPS [10] raised the consideration that LIT was due to chronic inhalation of the contaminating endotoxin. The present study was designed to assess the progression from AAD to LIT in endotoxin-resistant mice, in order to determine if chronic inhalation of endotoxin was required for the development of LIT.

To address this question, we obtained adult wildtype C57BL/6J (WT) and endotoxin-resistant C57BL/10ScN (ER) mice from The Jackson Laboratory (Bar Harbor, ME). These ER mice are a TLR4 deletion-based knockout strain. Their use in this study assumed that the relevant actions of inhaled LPS in murine OVA asthma models are mediated through the Toll4

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receptor, as previously demonstrated [8]. The mice were sensitized to OVA by receiving 3 weekly intraperitoneal injections of 25 μg OVA (grade V; Sigma Chemical Co., St. Louis, MO) and 2 mg aluminum hydroxide (alum) suspended in 0.5 ml of saline [10]. One week after the last injection, the mice were exposed to 1% aerosolized OVA either for 1 h/day for 7 days (acute exposure; AAD group), or for 1 h/day for 7 days followed by 5 days/week for 4 weeks (chronic exposure; LIT group). The OVA aerosols were generated by a BANG nebulizer (CH Technologies, Westwood, NJ) into a 7.6-L, 48-port inhalation exposure chamber to which mice placed in plastic restraint tubes were attached (CH Technologies). Chamber airflow was 6 L/min, and aerosol particle size of OVA was monitored by gravimetric analysis with a Mercer cascade impactor (In-Tox Products, Moriarty, NM). The mass median aerodynamic diameter (MMAD) and geometric standard deviation were 1.4 and 1.6 μm, respectively. Airway responses to methacholine were assessed by whole-body barometric plethysmography performed 12 h after the third OVA aerosol in acute animals or during the final week of exposure in chronic animals [10]. Bronchoalveolar lavage (BAL) cell counts and serum OVA-specific IgE levels were determined at the time of sacrifice [10].

Relative to naive animals, OVA-sensitized mice exposed to 7 days of inhaled OVA challenges demonstrated marked airway inflammation, characterized by increased total BAL cells and increased numbers of eosinophils and lymphocytes (Fig. 1A). There was no difference in the degree or composition of the BAL cellular inflammatory response between the WT and ER mice. In contrast to naive mice that had no detectable OVA-specific IgE, WT and ER mice developed similar levels of anti-OVA IgE following the sensitization and inhaled challenges (15.4±0.5 μg/ml WT and 18.1±0.5 μg/ml ER; Fig. 1B). Baseline sensitivity to inhaled methacholine, obtained in the mice prior to inhaled OVA challenge), was also similar in WT and ER mice (p2 methacholine concentration 25.5±3.1 mg/ml WT and 24.0±2.6 mg/ml ER). The similar airway eosinophilia and serum IgE responses in WT and ER mice suggested that the endotoxin contaminating our commercial OVA solutions was not enough to modulate the development of AAD in our mice. We have previously determined that our mice may have inhaled 5–35 ng/day of endotoxin [10]. This dose is substantially lower than the microgram doses used by others to show inhibitory effects of endotoxin on the development of allergic airway responses [7,12].

Even though the airway eosinophilia and serum IgE responses were similar in the two groups of mice, only the WT mice developed increased sensitivity to methacholine. After 3 inhaled OVA aerosol challenges, the WT mice demonstrated a 2.6-fold increase in methacholine sensitivity (Fig. 1C), while the ER mice showed no change in responsiveness ($P=0.040$ vs. WT response). The failure of ER resistant animals to demonstrate airway hyperresponsiveness during AAD was also shown in endotoxin-resistant BALB/c mice lacking LPS binding protein [13]. Of interest, TLR2 and TLR4 receptors have recently been identified in mouse [14] and human airway smooth muscle [15]. Conceivably, stimulation of these receptors with a low level of inhaled endotoxin may be needed for the development of airways hyperreactivity during inhaled antigen challenge.

With 6-week exposure to OVA aerosols, airway inflammatory responses largely resolved (Fig. 1A). There was no difference in the degree or composition of the BAL cellular inflammatory response between the WT and ER mice. Airway eosinophilia was completely ablated in both groups of mice. Airway lymphocytosis was also greatly attenuated, although it remained mildly increased compared to naive mice. Serum levels of OVA-specific IgE remained elevated in chronic WT and ER mice (Fig. 1B). There were no differences between chronic WT and ER anti-OVA IgE levels; however, the levels of anti-OVA IgE in LIT mice were lower than those in AAD mice ($P=0.024$). Methacholine responsiveness was not repeated because the ER mice did not demonstrate heightened reactivity during AAD, and the increased responsiveness seen in WT mice during AAD returns to baseline with progression to LIT [10,11]. Thus, the progression of our model from AAD to LIT was also

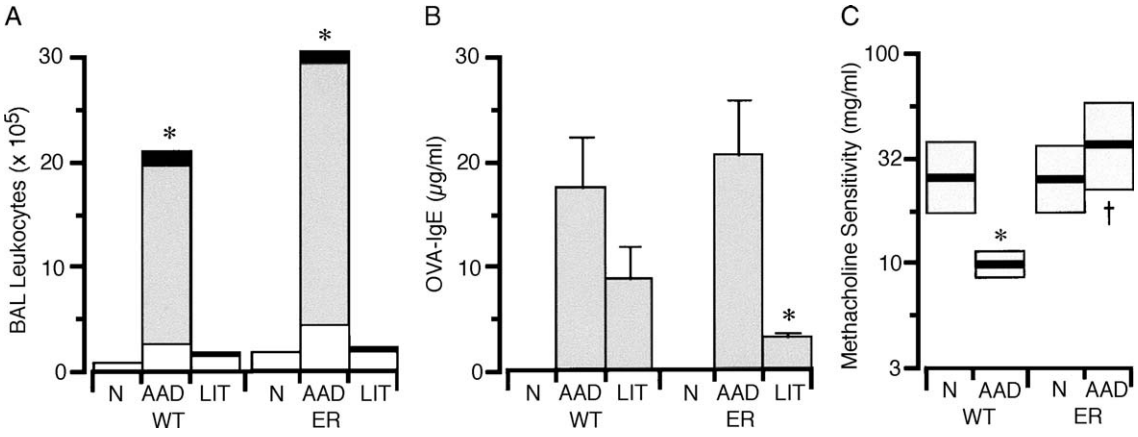

Fig. 1. Panel A: BAL leukocyte counts in naive, AAD, and LIT wildtype (WT) and endotoxin-resistant (ER) mice. Data represent mean values for macrophages (open bars), eosinophils (shaded bars), and lymphocytes (striped bars); * designates $P<0.05$ for total cells, eosinophils, and lymphocytes in AAD vs. corresponding naive mice. Panel B: Serum OVA-IgE levels in naive, AAD, and LIT WT and ER mice. Data represent mean±S.E.M. values; * designates $P<0.05$ for OVA-IgE levels between ER mice in the AAD and LIT phases. Panel C: Responsiveness to inhaled methacholine in naive and AAD WT and ER mice. Data represent mean±S.E.M. values; * designates $P<0.05$ vs. corresponding naive response, and † designates $P<0.05$ between WT and ER responses in similar conditions.

not mediated by the endotoxin contaminating the OVA solution, since chronic exposure of sensitized mice to aerosolized OVA resulted in the development of LIT in both WT and ER mice. Exposure of naive BALB/c mice to 40 ng/ml inhaled LPS before and during exposure to aerosolized OVA prevents the development of IgE tolerance to systemic OVA administration [9]; however, our model differs fundamentally from airway antigen-induced systemic tolerance in that LIT develops after AAD has been established and is localized to the lungs, with lesser effects on systemic responses.

In summary, the development of LIT with chronic aerosol exposure to OVA was independent of any potential effects of endotoxin in the OVA aerosol solution, or of endotoxin arising from cage litter and feces. In contrast, the development of hyperresponsiveness to methacholine during AAD appeared to be dependent on an intact endotoxin-signaling pathway, suggesting that endotoxin may contribute to the acute airway response in asthma.

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