

Biphasic Late Airway Hyperresponsiveness in a Murine Model of Asthma

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Key Words

Asthma • Biphasic late airway hyperresponsiveness • Tumor necrosis factor • Cytosolic phospholipase A₂ • Leukotriene B₄

Abstract

Background: Nonspecific airway hyperresponsiveness (AHR) is one of the cardinal features of bronchial asthma. Early AHR is caused by chemical mediators released from pulmonary mast cells activated in an IgE-dependent way. However, the mechanism of late AHR remains unclear. **Methods:** Features of airway allergic inflammation were analyzed, including antigen-induced AHR, using a murine model of asthma. The model was suitable for examining the sequential early molecular events occurring after the initial airway exposure to antigen. **Results:** AHR increased at 10–12 h after airway challenge, followed by the second-phase response, which was larger and broader in resistance at 18–30 h. Pretreatment of sensitized animals with anti-tumor necrosis factor (TNF) before airway challenge or induction of allergic asthma in TNF^{−/−} mice resulted in abrogation of the first-phase late AHR. Intratracheal instillation of TNF induced a single peak of AHR at 10 h. IgE and IgG immune complexes induced the development of the first-phase late AHR by TNF

production. Pretreatment with cytosolic phospholipase inhibitor and 5-lipoxygenase inhibitors abolished the first-phase late AHR as well as the leukotriene B₄ levels in the airway. CpG-oligodeoxynucleotide (ODN) pretreatment reduced airway levels of Th2 cytokines, eosinophil infiltration and second-phase late AHR. However, CpG-ODN did not reduce TNF levels or the magnitude of first-phase late AHR. **Conclusion:** Biphasic late AHR occurs in a murine model of asthma. First- and second-phase late AHR is caused by TNF and Th2 response, respectively.

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Introduction

Nonspecific airway hyperresponsiveness (AHR) is one of the cardinal features of bronchial asthma. When allergic asthmatics are exposed to the antigen, they develop an acute IgE-dependent early AHR, which is often followed by late AHR from 4 to 12 h after the exposure [1, 2]. The early reaction is thought to be caused by many chemical mediators released from pulmonary mast cells

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activated in an IgE-dependent way [3]. It is generally accepted that cytokines secreted by Th2 cells play a critical role in the development of late AHR although it has often been proposed that CD4⁺ T cells promote the development of late AHR indirectly through the recruitment of eosinophils and/or other leukocytes [4]. Interleukin (IL)-4, IL-5, IL-9, and IL-13 are believed to be the key cytokines involved in the production of IgE for the activation of mast cells, as well as for the induction of bronchial hyperresponsiveness, lung eosinophil infiltration, and mucus secretion [5, 6].

Tumor necrosis factor (TNF) is an important cytokine in the innate immune response, which plays a key role in the immediate host defense against invading microorganisms before activation of the adaptive immune system [7]. The possibility that TNF contributes to the inflammatory response seen in the asthmatic airway is supported by observations that TNF mRNA [8] and protein [9] levels are increased in the airways of patients with asthma. Importantly, the administration of inhaled recombinant TNF to normal subjects leads to the development of AHR [10, 11]. We have recently described TNF-induced late AHR in a murine model of asthma [12, 13]. In the model, mice received two intraperitoneal immunizations, followed by two bronchoprovocations with aerosolized ovalbumin (OVA) with a 1-week interval. Using this protocol, we described early and late AHR occurring at 1 h and 10–14 h after the second airway allergen challenge, and the late AHR was caused by TNF produced in the airway, which, in turn, promoted the activation of the enzyme, cytosolic phospholipase A₂ (cPLA₂). Collectively, the information suggests that TNF and Th2 responses are important factors for inducing late AHR.

Presently, we investigated whether the late AHR could be discerned using this murine model of allergic asthma. Two peaks of late AHR were evident, which were dependent on TNF and Th2 responses.

Materials and Methods

Mice

Specific pathogen-free female C57BL/6 mice purchased from Orient Bio (Gapyeong, Gyeonggi, Korea) were housed in a laminar flow cabinet and maintained on standard laboratory chow ad libitum. TNF knock-out mice (TNF^{-/-}) derived from breeder pairs of B6; 129S-Tnfr1Gk1 stock and the original wild-type strain (B6129SF2/J) were obtained from the Jackson Laboratory (Bar Harbor, Me., USA). Mice were 7–8 weeks old at the start of each experiment. All mice used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the Chonbuk National University Medical School.

Reagents

OVA (grade V) was purchased from Sigma-Aldrich (St. Louis, Mo., USA). The cPLA₂ inhibitor, arachidonyltrifluoromethyl ketone (AACOCF₃; Merck KGaA, Darmstadt, Germany), was administered intraperitoneally (20 mg/kg) 30 min before the second airway challenge [14]. The 5-lipoxygenase (5-LO) inhibitors AA-861 and MK-886 were purchased from Sigma-Aldrich and Cayman Company (Ann Arbor, Mich., USA), respectively. AA-861 (2 mg/kg) was administered intraperitoneally 1 h before the second airway challenge [15]. MK-886 (250 µg/kg) was administered subcutaneously 1 h before the second airway challenge [16]. The optimal concentrations of the inhibitors were determined in preliminary experiments. AACOCF₃ and AA-861 were dissolved in ethanol and MK-886 was dissolved in DMSO. Working solutions contained 5% of each vehicle.

Induction of Murine Asthma

Mice were immunized intraperitoneally with 20 µg of OVA plus 1.0 mg aluminum hydroxide adjuvant on day 0 and OVA alone on day 10 [12, 13]. The immunized mice were exposed to aerosolized OVA on days 17 and 24. Aerosolization of OVA was performed using a chamber adapted for mice. Animals were exposed to OVA (2.0% at first aerosolization and 4.0% at second aerosolization) using a NE-U12 ultrasonic nebulizer (Omron, Tokyo, Japan) at an output of 0.8 ml/min for 20 min in a Plexiglas exposure chamber (24.5 × 40.5 × 15.0 cm). Control animals received the same immunization and the first airway challenge of OVA but were exposed to aerosolized saline instead of OVA at the second airway challenge.

IgE and IgG Immune Complexes

Mouse IgG1 monoclonal antibody specific for chicken γ-globulin (CGG) was prepared as ascites in pristane-primed nude mice [17]. The IgG fraction was further purified using a protein G column (Sigma-Aldrich). CGG immune complex (IC) was prepared at twofold antigen excess, based on equivalence points determined by quantitative precipitin curves. CGG IC was incubated for 2 h at 37°C and thereafter overnight at 4°C. After that, ICs were washed three times (500 g/min, 10 min), resuspended in cold PBS and stored at 4°C. Anti-DNP IgE (1 µg) (Sigma-Aldrich) was administered Intratracheally, followed by intratracheal administration of 2 µg of DNP-BSA (Sigma-Aldrich) 1 day later [18].

Intratracheal Administration

Mice were anesthetized using 80 mg/kg of pentobarbital sodium injected intraperitoneally and placed on a vertical platform. Intratracheal administration was performed using a 50-µl glass syringe (Hamilton, Reno, Nev., USA) fitted with a 2-inch, 26-gauge blunt-end needle. The needle was inserted directly above the trachea.

Bronchoalveolar Lavage

Bronchoalveolar lavage (BAL) was performed as described previously [12, 13].

Determination of AHR

AHR was also assessed as a change in airway function after challenge with aerosolized methacholine via airways as described elsewhere [19, 20]. Anesthesia was achieved with 80 mg/kg of pentobarbital sodium injected intraperitoneally. The trachea was ex-

posed by mid-cervical incision. Mice were tracheostomized and an 18-gauge metal needle was then inserted. Mice were connected to a computer-controlled small-animal ventilator (flexiVent, Scireq, Montreal, Que., Canada). The mouse was quasi-sinusoidally ventilated with a nominal tidal volume of 10 ml/kg at a frequency of 150 breaths/min and a positive end-expiratory pressure of 2 cm H₂O to achieve a mean lung volume close to that attained during spontaneous breathing. This was achieved by connecting the expiratory port of the ventilator to a water column. Methacholine aerosol was generated with an in-line nebulizer and administered directly through the ventilator. To determine the differences in airway response to methacholine, each mouse was challenged with methacholine aerosol at increasing concentrations (2.5–50 mg/ml in saline). After each methacholine challenge, the airway resistance (R_L) data were continuously collected. Maximum values of R_L were selected to express changes in airway function, which was represented as a percentage change from baseline after aerosol saline challenge. We used different groups of mice to assess AHR at different time points. In some cases, we presented the changes of R_L at 50 mg/ml of methacholine.

Measurement of LTB₄

The level of LTB₄ in BAL fluid was quantified using ELISA performed according to the protocol of the manufacturer (R&D Systems, Minneapolis, Minn., USA). Sensitivity was 3.7 pg/ml.

ELISA

TNF, IL-4, IL-5, IFN- γ , keratinocyte-derived chemokine (KC), and macrophage inflammatory protein (MIP)-2 protein levels in BAL fluids were determined by ELISA. The lower limits of detection for the cytokines were as follows: TNF (>5.1 pg/ml; R&D Systems), IL-4 (>5 pg/ml; Endogen, Cambridge, Mass., USA), IL-5 (>5 pg/ml; Endogen), IFN- γ (>0.7 pg/ml; BD Biosciences, Franklin Lakes, N.J., USA), KC (>2 pg/ml; R&D Systems), and MIP-2 (>1.5 pg/ml; R&D Systems).

Treatment with Anti-TNF

Two milligrams of anti-TNF (MP6-XT22.11) [7] and control rat IgG1, J4-1 [21] were injected intraperitoneally 10 min before the IgG IC instillation.

Immunoblotting Analysis

Immunoblotting analysis was performed to confirm that p50 small interfering RNA blocked expression of its target protein. Mice were sacrificed by cervical dislocation and the lungs were collected immediately thereafter, briefly washed with cold PBS and dried with blotting paper. The isolated lung tissues were frozen in liquid nitrogen and were stored in -70°C until analysis. Small lung specimens were homogenized in the PhosphoSafe Extraction Reagent (Novagen, Madison, Wisc., USA) and were subjected to immunoblotting analysis as described previously [13].

CpG-Motif Oligodeoxynucleotides

The CpG oligodeoxynucleotides (CpG-ODN) consisted of 20 bases containing two CpG motifs (TC CATGACGTTCTGACGTT). The control ODN was identical except that the CpG motifs are rearranged, as denoted in italics (TCCATGAGCTTCCTGAGTCT). ODN were produced by Oligos Etc. (Wilsonville, Oreg., USA) in a Good Manufacturing Practice facility and had

undetectable levels of lipopolysaccharide. ODN (30 μg) were administered by intraperitoneal injection on day 0 [22].

Statistical Analyses

Data are expressed as means $\pm$ SDs. Statistical comparisons were performed using one-way ANOVA followed by the Fisher exact test. Significant differences between groups were determined using the unpaired Student *t* test. $p < 0.05$ was considered statistically significant.

Results

Occurrence of Biphasic Late AHR in Murine Asthma

We examined the kinetics of late AHR up to 48 h at 2- to 6-hour intervals after secondary airway allergen challenge. Methacholine challenge (50 mg/ml) increased R_L by 4.0- to 4.5-fold at 10–12 h, followed by the second-phase response, which was larger and broader in resistance at 18–30 h, peaking at around 24 h with a 5-fold increase (fig. 1a), indicating that biphasic late AHR occurred. The time course study of the BAL levels of TNF and Th2 cytokines revealed that TNF reached a peak within 1.5–2 h but rapidly declined thereafter and returned to the basal level throughout the experiment. Th2 cytokines such as IL-4, IL-5, and IL-13 peaked at 18 h and declined thereafter (fig. 1b). We also assessed cell numbers in the airway. The cellular infiltrate is initially dominated by neutrophils, with an increasing infiltration of macrophages, lymphocytes and eosinophils (fig. 1c).

TNF Is Responsible for the Development of First-Phase Late AHR

Pretreatment of the sensitized animals with anti-TNF before airway challenge or induction of allergic asthma in TNF $^{-/-}$ mice resulted in abrogation of the first phase of late AHR measured at 12 h (fig. 2a). Furthermore, intratracheal instillation of TNF induced a single peak of AHR at 10 h (fig. 2b), but not at 24 h, in response to 50 mg/ml methacholine challenge. These data indicated that the first-phase late AHR is caused by TNF.

Effects of IgE and IgG ICs on the Development of the First-Phase Late AHR

TNF has been reported to be produced during allergic responses by inflammatory cells such as mast cells and macrophages via an IgE-dependent way [23]. In this study, as a mechanism leading to the first-phase late AHR, we compared the abilities of IgE and IgG ICs to induce TNF synthesis in the airway and late AHR. A single intratracheal instillation of IgE IC (anti-DNP IgE + DNP-

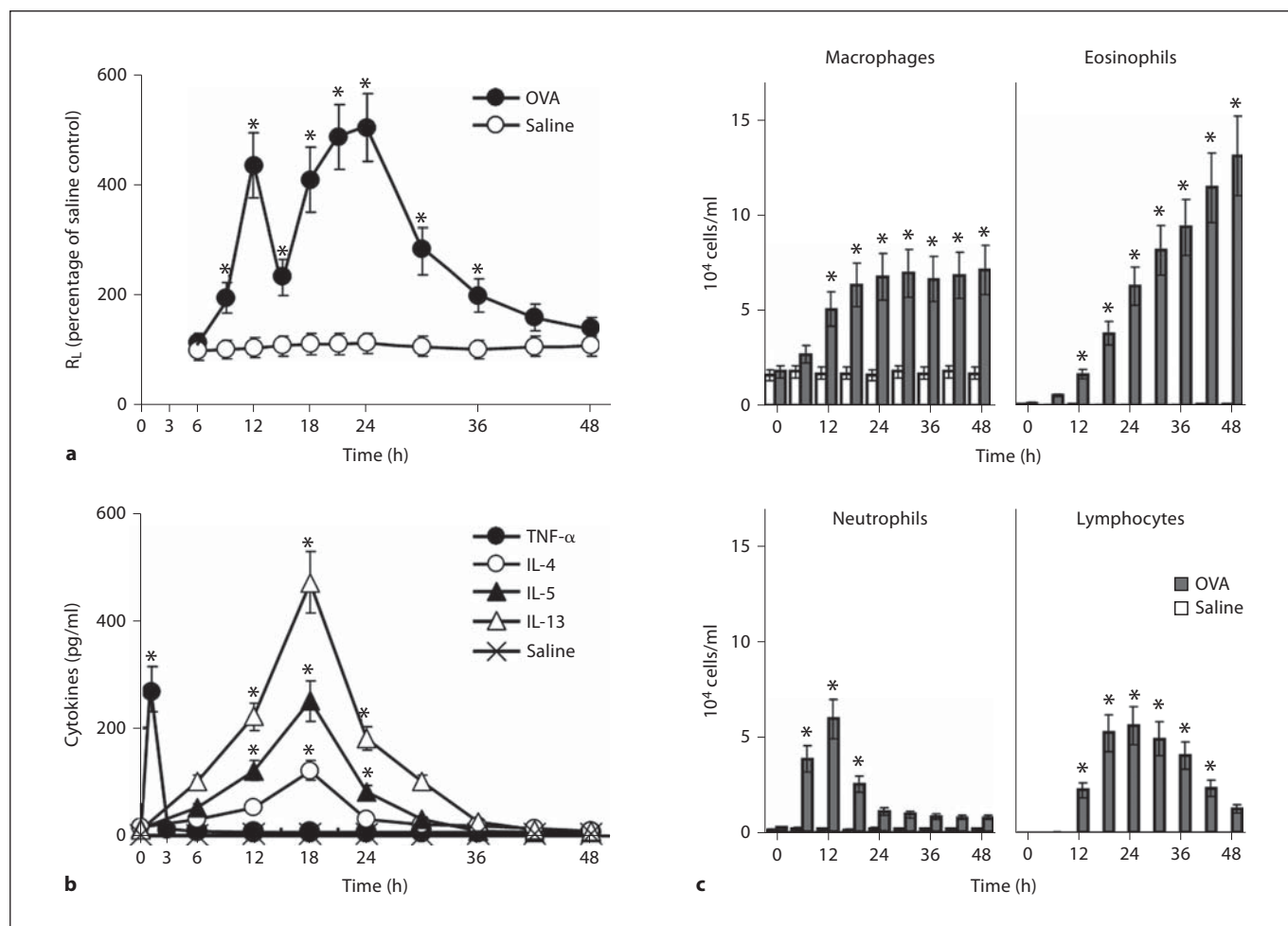


Fig. 1. Occurrence of biphasic late AHR. **a** Lung resistances were measured in different groups of mice at the times indicated after the second airway challenge. Cytokines in BAL fluids (**b**) and the numbers of inflammatory cells in the airway (**c**) were determined at the times indicated after second airway challenge. Data represent the means $\pm$ SDs of 3–7 separate experiments ($n = 5$ per group). * $p < 0.05$ vs. saline.

BSA) (fig. 3a) or IgG IC (anti-CGG IgG1 monoclonal antibody + CGG) (fig. 3b) resulted in a dose-dependent increase in the BALF levels of TNF. The capability of IgG IC to produce TNF was 3- to 4-fold higher than that of IgE IC. They also induced AHR at 12 h (fig. 3c), but there was no further development of AHR beyond this time point, i.e. at 24 or 36 h (data not shown). The IgE IC- or IgG IC-mediated AHR did not occur in TNF^{-/-} mice (fig. 3c). Taken together, these results indicated that the first-phase late AHR mediated by TNF originated from the airway residential cells with receptors for allergen-specific IgE and IgG.

cPLA₂/5-LO/LTB₄ Pathway Is Involved in TNF-Induced Late AHR

We previously reported that TNF induces late AHR by enhancing phosphorylation and enzymatic activation of cPLA₂ [12, 13]. However, the downstream pathway of cPLA₂ remains to be elucidated. In this study, we investigated the role of LTB₄ in this process, as among cPLA₂/5-LO metabolites, LTB₄ plays a key role in the development of AHR [24–27].

Pretreatment with the cPLA₂ inhibitor AACOCF₃ abolished the first-phase late AHR (fig. 4a). The AHR was also significantly diminished by pretreatment with the 5-LO inhibitors AA-861 or MK-886 (fig. 4a), suggesting that the 5-LO metabolite plays a role in the develop-

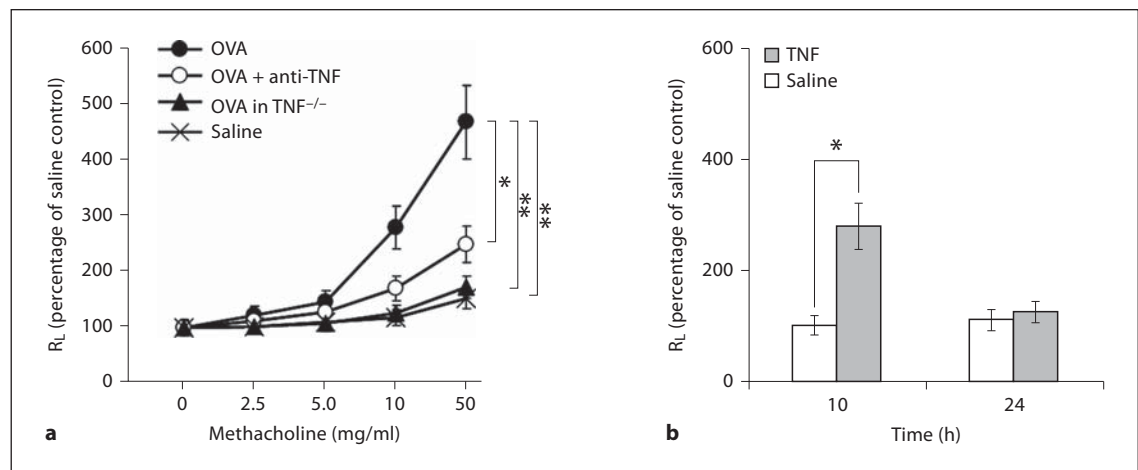


Fig. 2. TNF induces first-phase late AHR. **a** AHR was measured 12 h after the second airway challenge in TNF^{-/-} mice and in mice pretreated with anti-TNF. **b** AHR was measured 10 and 24 h after intratracheal instillation of TNF (0.3 µg) into normal mice. Data represent the means ± SDs of 2 or 3 separate experiments (n = 5 per group). * p < 0.05, ** p < 0.01.

ment of TNF-induced late AHR. Therefore, we evaluated the role of LTB₄ in TNF-induced late AHR. Our kinetic study revealed that increases in BAL levels of LTB₄ occurred after the second airway challenge, peaking at 1–2 h, and pretreatment of AACOCF₃, AA-861 or MK-886 resulted in the inhibition of the increase of LTB₄ levels in asthmatic mice (fig. 4b). The vehicle (ethanol or DMSO) itself did not affect the BAL levels of LTB₄ in OVA-sensitized/challenged mice (data not shown). Intratracheal instillation of TNF in normal mice resulted in increases in LTB₄ in the airway (fig. 4c), and AA-861 or MK-886 inhibited the TNF-induced AHR (fig. 4d). Taken together, these data suggest that the cPLA₂/5-LO/LTB₄ pathway is critical in the development of TNF-induced late AHR.

CpG-ODN Abolishes Second-Phase but Not First-Phase Late AHR

Given that Th2 cell-derived cytokines are also critical for the induction of late AHR, we examined the association of late AHR with the Th2 response. We assessed this connection using CpG-ODN as it is well established that CpG-ODN inhibits Th2 cell-mediated phenotypes by inducing a Th1 response in murine asthma models [22, 28, 29]. We verified that CpG-ODN inhibited Th1 as well as Th2 responses. CpG-ODN pretreatment, but not control CpG pretreatment, significantly reduced BALF levels of IFN-γ, IL-4, IL-5 and IL-13 (fig. 5a) as well as airway eosinophilia (fig. 5b). However, rather than reducing TNF

levels in BAL fluids, CpG-ODN increased them approximately 2-fold (fig. 5c). CpG-ODN, but not control CpG, abolished the second phase of late AHR at 24 and 30 h, but it did not reduce the magnitude of first-phase late AHR in response to a 50 mg/ml methacholine challenge (fig. 5d). These data indicate that the second-phase late AHR is caused by the Th2 response.

Discussion

Usually, the protocols which have been employed to study asthma in mice comprise a double sensitization with antigen mixed with an adjuvant such as alum, followed by airway antigen challenge for more than 3 consecutive days, and examination of AHR and lung inflammation 1–3 days after the last antigen challenge [30–34]. However, these protocols do not allow examination of early events occurring after the initial airway exposure to antigen because the early and the late responses overlap and become almost indistinguishable as the response expands. In this study, we used the previously developed protocol in which mice were immunized twice intraperitoneally, followed by two bronchoprovocations with aerosolized OVA at a 1-week interval. This model seems to be a suitable tool to identify the cascade of events involved in the asthmatic reaction.

Using this protocol, we were able, for the first time, to reveal a biphasic late AHR, peaking at 12 and 24 h.

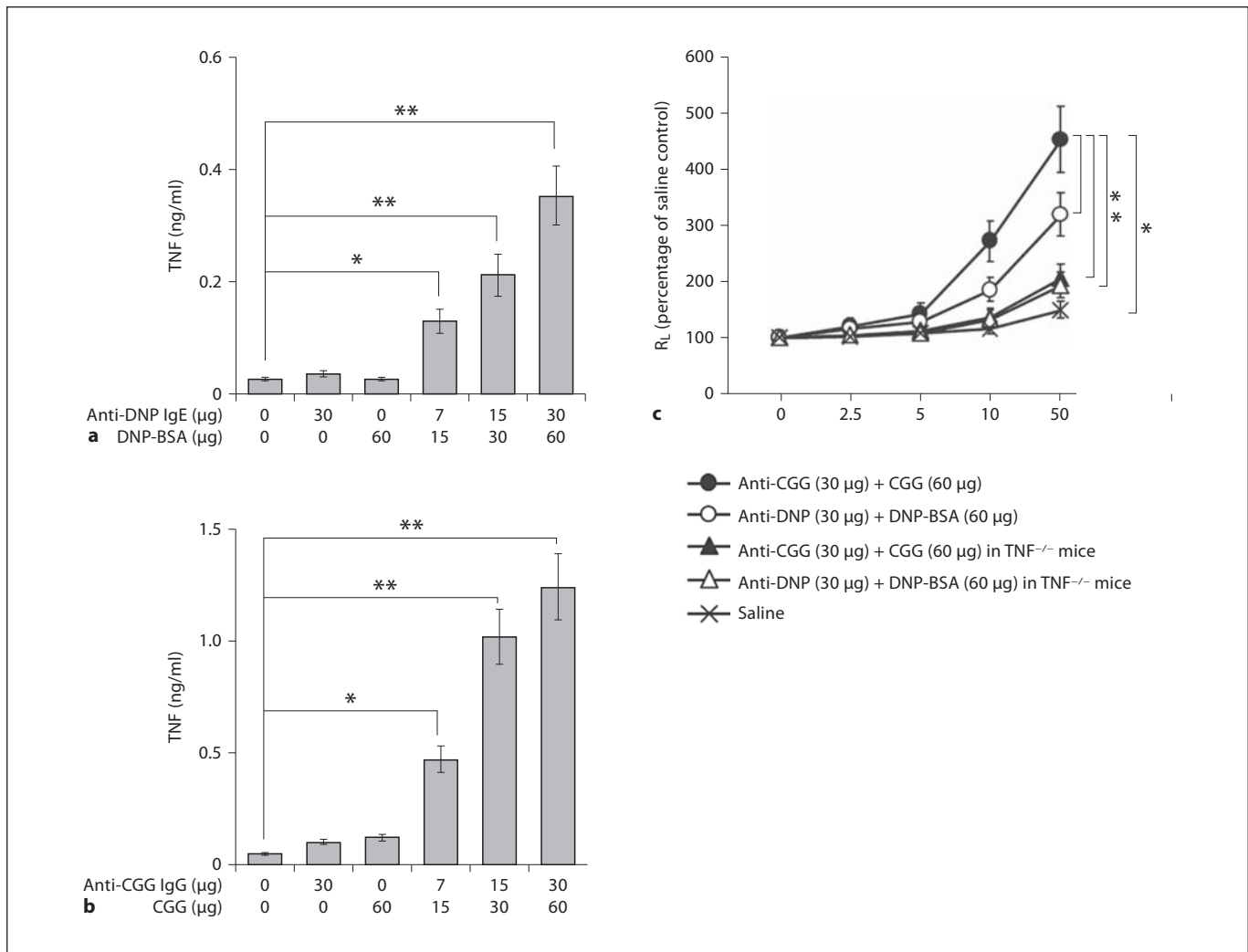


Fig. 3. Both IgE and IgG ICs cause first-phase late AHR via TNF. BAL fluid levels of TNF were measured 1.5 h after a single intratracheal instillation of varying concentrations of IgE IC (**a**) or IgG IC (**b**) in normal mice. **c** AHR was measured 12 h after a single intratracheal instillation of IgE IC or IgG IC into normal and TNF^{-/-} mice. Data represent the means $\pm$ SDs of 2 or 3 separate experiments ($n = 5$ per group). * $p < 0.05$, ** $p < 0.01$.

We have shown the contribution of TNF to the development of antigen-induced late AHR at 10–12 h [12, 13]. In this study, we confirmed that the late AHR corresponded to the first-phase late AHR in this study by demonstrating that the first-phase late AHR in asthmatic mice was abolished either by pretreatment with anti-TNF or in asthmatic TNF^{-/-} mice (fig. 2a) and that the intratracheal instillation of TNF resulted in a single AHR at 10 h (fig. 2b). The present study further extends our efforts to reveal the molecular mechanisms leading to TNF production in asthmatic lungs. We investigated the role of IgE and IgG antibodies by intratracheal instilla-

tion of IgE and IgG ICs. A single intratracheal injection with ICs resulted in an increase in TNF in BAL fluids (fig. 3a, b). Twice as much TNF was produced in response to IgG IC. Furthermore, both ICs were capable of inducing AHR at 12 h, which was abolished either by pretreatment with anti-TNF or in asthmatic TNF^{-/-} mice. These data indicate that IgE and IgG antibodies are capable of inducing late AHR via the production of TNF in the airway.

Among the cell populations capable of producing TNF in the airway, mast cells represent a potential source of TNF [35, 36]. In fact, mast cell-derived TNF plays a key

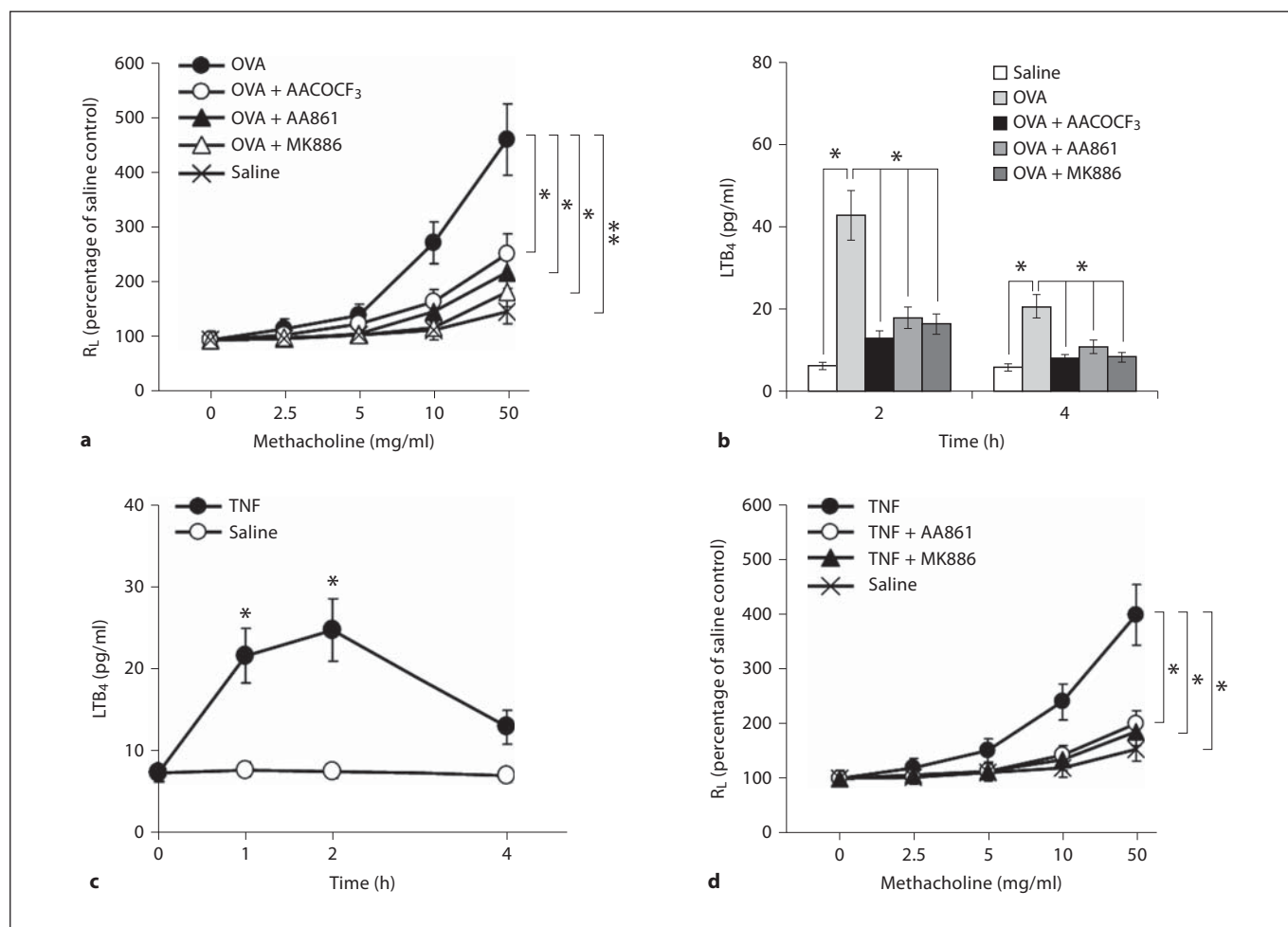


Fig. 4. The cPLA₂/5-LO/LTB₄ pathway is involved in TNF-induced late AHR. The first-phase late AHR at 12 h (**a**) and BAL fluid levels of LTB₄ (**b**) in asthmatic mice were measured. Mice were pretreated with cPLA₂ inhibitor AACOCF₃ or 5-LO inhibitors AA861 and MK886 before the second airway challenge as described in Materials and Methods. **c** BAL fluid levels of LTB₄ were measured after intratracheal instillation of TNF (0.3 µg) into normal mice. **d** AHR was measured 12 h after intratracheal instillation of TNF (0.3 µg) into normal mice. Mice were pretreated with 5-LO inhibitors 30 min before TNF instillation. Data represent the means ± SDs of 2 or 3 separate experiments (n = 5 per group). * p < 0.05, ** p < 0.01.

role in the development of late AHR [12, 37]. Murine mast cells not only express high-affinity receptors for IgE (FcγRI), but they also express low-affinity receptors for IgG [38] which are of the FcγRII and FcγRIII types [39, 40]. As a result, mast cells produce TNF in response to cross-linking of not only FcγRI but also FcγR through the IgG IC [41, 42]. The TNF-producing capability of IgG IC was superior to that of IgE IC (fig. 3c). This may be explained by the fact that, in contrast to IgE which stimulates only mast cells in the mouse airway [43], IgG can also stimulate other inflammatory cells such as macrophages, dendritic cells, eosinophils and platelets, which

express FcγRs on their cell surfaces [44]. Taken together, our results indicate that initial stimuli leading to TNF synthesis in the airway involve interactions between antigen and antibody on innate immune cells. TNF that is produced as a result of these actions induces AHR corresponding to the first-phase late AHR in this murine model of asthma.

The mechanisms driving TNF-induced AHR have not been fully elucidated. We have reported that cPLA₂ is crucial in TNF-induced late AHR [12, 13]. In order to clarify the underlying mechanism by which cPLA₂ exerts such an action, we assessed the role of LTB₄ in this study.

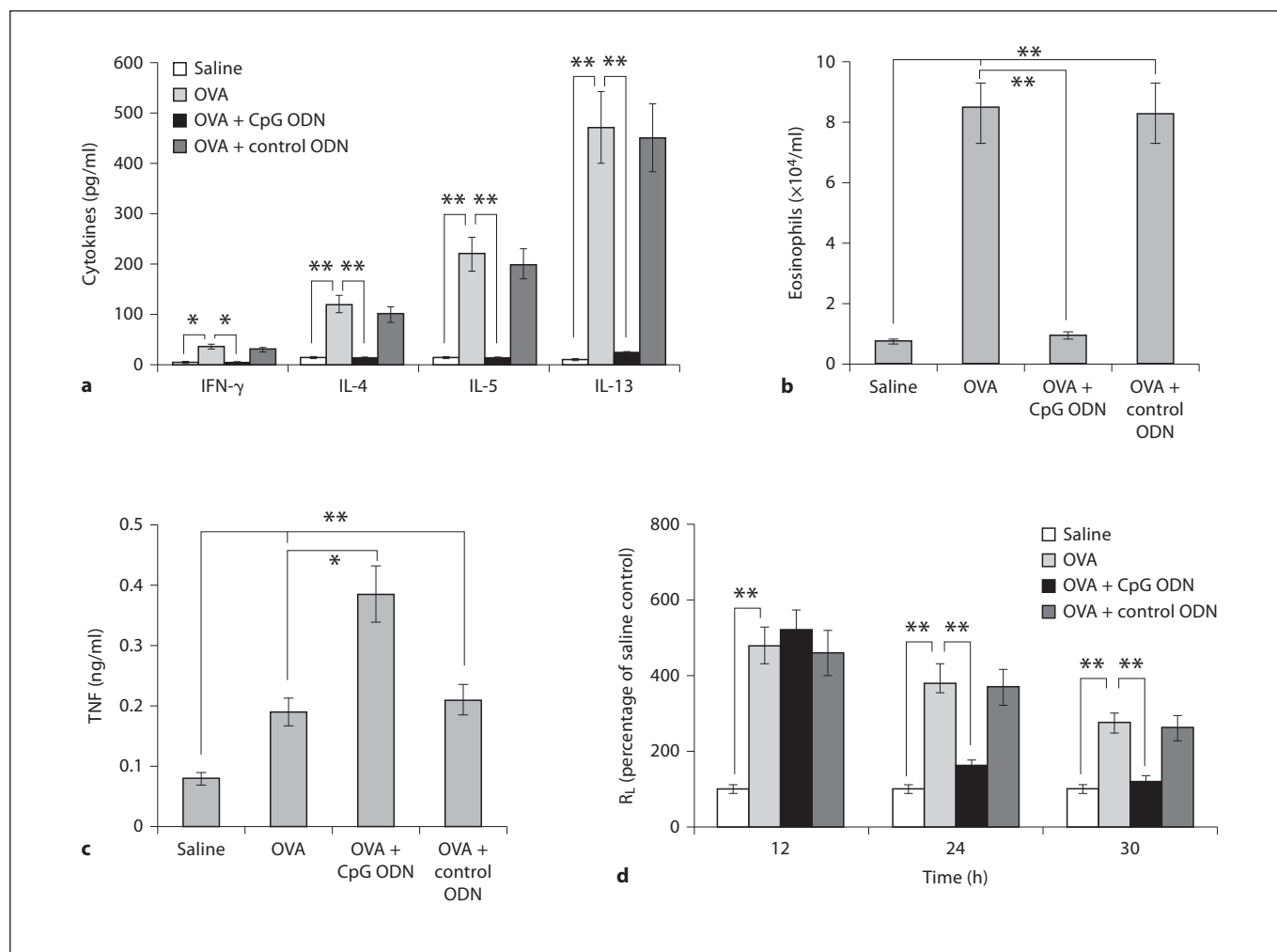


Fig. 5. CpG-ODN abolishes second-phase late AHR. Mice received a second airway challenge. BAL fluid levels of IFN- γ were measured at 12 h and Th2 cytokines (IL-4, IL-5 and IL-13) at 18 h (**a**); BAL fluid eosinophils were counted at 48 h (**b**), and BALF levels of TNF were measured at 1.5 h (**c**) were measured, AHR was measured 12, 24, and 30 h after the second airway challenge (**d**). CpG-ODN and control ODN were administered as described in Materials and Methods. Data represent the means $\pm$ SDs of 2 or 3 separate experiments ($n = 5$ per group). * $p < 0.05$, ** $p < 0.01$.

LTB₄ is derived from membrane phospholipids by the sequential action of cPLA₂, 5-LO and LTA₄ hydrolase [45, 46]. LTB₄ is a chemoattractant for leukocytes, including neutrophils, macrophages, monocytes and eosinophils [47–50]. Furthermore, it has been shown to be critical in the development of AHR [25, 51–53]. Presently, we demonstrated that cPLA₂ inhibitor and 5-LO inhibitors inhibit not only first-phase late AHR but also BAL the levels of LTB₄ that is formed after a second airway challenge. These data strongly suggest that TNF induces late AHR via LTB₄ production through the activation of the cPLA₂/5-LO pathway.

TNF can induce AHR by directly stimulating airway smooth muscle (ASM) contractile function. The small monomeric G protein rhoA can enhance ASM contractility to any agonist by increasing the levels of myosin light-chain phosphorylation through the rho-activated kinase-dependent suppression of myosin light-chain phosphatase [54]. Importantly, this rho-dependent calcium sensitization can be activated by TNF [55]. However, it seems unlikely that TNF induces AHR by directly stimulating ASM in this acute form of murine asthma because the time interval between the peak BAL level of TNF and the time of first-phase late AHR is around 10 h and be-

cause most of the first-phase late AHR was abrogated by pretreatment with cPLA₂ inhibitor or 5-LO inhibitor. However, LTB₄ may be able to induce ASM contractility by modifying its contractile susceptibility.

To identify the mechanism underlying the development of second-phase late AHR, we tried to suppress Th2 responses using CpG-ODN as it is well established that CpG-ODN inhibits Th2 cell-mediated phenotypes by inducing a Th1 response in murine asthma models [28, 29]. CpG-ODN markedly suppressed Th1 (IFN- γ) and Th2 phenotypes such as airway eosinophilia as well as the BAL fluid levels of IL-4, IL-5 and IL-13, but it also suppressed second-phase late AHR (fig. 5a, d). However, CpG-ODN did not inhibit TNF levels in BAL fluid or the magnitude of the first-phase late AHR. These data indicate that the second-phase late AHR is a Th2 response-dependent process and that the inhibition of the Th2 response does not result in inhibition of the TNF-induced first-phase late AHR. Therefore, although it is well established that allergen-specific Th2 cells and their resultant production of cytokines such as IL-4, IL-5 and IL-13 are critical for the development of late AHR, targeting just the Th2 response does not seem to be sufficient to control late AHR.

A number of studies have suggested a role for TNF in the development of AHR. Inhalation of TNF by animals [56, 57] and healthy [58] or asthmatic [59] human subjects leads to AHR. In murine models of asthma, either a deficiency in TNF receptors [60] or induction of a TNF auto-

antibody [61] results in marked attenuation of antigen-induced airway inflammation and AHR. Recent studies have demonstrated that anti-TNF therapy in patients with asthma can markedly improve asthma symptoms, lung function, and AHR, in which the most striking feature of anti-TNF therapy is the improvement in AHR [62, 63], further indicating a key role of TNF in the development of AHR.

The main finding of this study is that two phases of late AHR can be elicited in a murine model of asthma; these two phases are regulated by different mechanisms, i.e. TNF derived from residential cells in the airway upon antigen/antibody reaction and Th2 response. This study provides important information not only to improve our understanding of the mechanisms underlying AHR but also to establish therapeutic strategies for late AHR.

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Disclosure Statement

The authors declare no financial or commercial conflict of interest.

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