

Dual Role of Interleukin-1 α in Delayed-Type Hypersensitivity and Airway Hyperresponsiveness

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Key Words

Interleukin-1 · Allergic asthma · Th1/Th2

Abstract

Background: Using a T helper (Th)1/Th2 disease model, we previously showed that genetically determined Th development depends on dendritic cell-derived interleukin (IL)-1 α . In *Leishmania major* infections, Th1 immunity develops if IL-1 α is present during T cell priming, whereas at later time points, IL-1 α worsens disease outcome. In the present study, we determined the role of IL-1 α in other Th2-mediated diseases. **Methods:** BALB/c mice were subjected to delayed-type hypersensitivity (DTH) or ovalbumin (OVA)/alum-induced allergic asthma in the presence or absence of IL-1 α . **Results:** In DTH, mice treated with IL-1 α during sensitization with keyhole limpet hemocyanin (KLH)/alum developed decreased footpad swelling associated with elevated KLH-specific interferon- γ levels. In asthma, significantly decreased airway hypersensitivity responses (AHRs) were detected upon treatment with IL-1 α during T cell priming. In contrast to control mice, IL-1 α -treated mice showed reduced peribronchial inflammatory infiltrates. The bronchoalveolar lavage (BAL) fluid contained significantly decreased eosinophil numbers (approximately 50%), but 4 times more neutrophils. The BAL fluid of IL-1 α -treated BALB/c exhibited reduced amounts of IL-5 and OVA-specific IgE serum levels.

In contrast, IL-1 α treatment at later time points after sensitization or during allergen challenge worsened AHR, had no effect on lung inflammation and BAL fluid cell composition. Furthermore, cytokine levels (IL-5, IL-13) and antigen-specific IgE were increased or unaltered under these conditions. **Conclusion:** Similarly to leishmaniasis, IL-1 α administration during sensitization of Th2-mediated allergic reactions suppresses the course of disease by shifting the immune response towards Th1, whereas later treatments worsen disease outcome. Future studies will elucidate the therapeutic value of IL-1 α in asthmatic patients.

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Introduction

For many years, murine cutaneous leishmaniasis has been studied extensively as a disease model for the T helper (Th)1/Th2 paradigm [1]. In leishmaniasis, factors intrinsic to dendritic cells (DCs) are important for regulating resulting Th immune responses [2–4]. Protective immunity as observed in most mouse strains (including C57BL/6) depends on the development of interferon (IFN)- γ -producing Th1/Tc1 cells. In contrast, BALB/c mice develop progressive disease due to Th2 immune responses.

Interestingly, DCs derived from genetically *Leishmania*-resistant and -susceptible mice preferentially either induced Th1 and Th2 responses in CD4⁺ T cells, respectively [2]. Decreased IL-1 β released from activated BALB/c DCs appeared to be responsible for this difference [2]. In support, antigen-presenting cell-derived IL-1 α and TNF- α together with IL-12 was required to induce efficient Th1 priming in BALB/c CD4⁺ T cells, whereas C57BL/6 cells required only IL-12 [5]. Independently, we found that *Leishmania major*-infected DCs from BALB/c mice produced significantly less IL-1 α (and β) than DCs from C57BL/6 mice [3]. We demonstrated that treatment of susceptible BALB/c mice with IL-1 α during Th development induced Th1-mediated protection. In contrast, prolonged treatment with IL-1 α (for 3 weeks) worsened disease outcome in cutaneous leishmaniasis [6].

Our data suggested that IL-1 engages different Th subpopulations at different times after infection. In early phases of *L. major* infections, IL-1 facilitated Th1 development from Th0 cells, whereas later on high-affinity IL-1 receptor type I (IL-1RI) signaling promoted Th2 expansion and worsened disease outcome. Previously, some studies suggested that IL-1 favors the expansion of Th2, but not Th1 clones [5, 7–9]. IL-1 activated Th2 cells and thus enhanced antibody production [8, 10–13]. Other data indicated that IL-1 was not required for Th2 differentiation [14]. Interestingly, naive Th0 and Th2, but not Th1, cells expressed IL-1RI *ex vivo* [6]. Thus, different levels of IL-1RI expression by various Th populations may be responsible for the different results.

Taken together, the exact role of IL-1 in the regulation of Th immunity is still unclear. Interestingly, differential expression of IL-1 α/β by BALB/c and C57BL/6 DCs was observed not only after infection with *L. major* but also when the cells were stimulated with other agents, e.g. lipopolysaccharide [2, 3]. Thus, in the present study, the effects of IL-1 α on Th development were tested using two other models known to induce Th2-dependent immunity: (a) keyhole limpet hemocyanin (KLH)/alum-mediated delayed-type hypersensitivity (DTH) [15, 16], and (b) ovalbumin (OVA)/alum-induced airway hypersensitivity in mice, a relevant model for human allergic asthma [17]. We will show that IL-1 α administration during T cell priming induced preferential Th1 immunity in these models associated with decreased footpad swelling in DTH and improved airway resistance in allergic asthma, respectively. In contrast, if IL-1 α was administered at later stages after induction of airway hypersensitivity when OVA-specific Th2 responses were already estab-

lished, disease outcome was unaltered or even worse. Taken together, our data indicate that the timing of IL-1 α administration during allergic asthma is critical for disease outcome.

Materials and Methods

Animals

Female wild-type BALB/c mice, 6–10 weeks old, were bred and housed under specific pathogen-free conditions in the Animal Care Facility in Mainz in accordance with institutional and federal guidelines. All experiments were undertaken with approval from the Animal Care and Use Committee of the Federal State Rheinland-Pfalz.

Induction of DTH

On day 0, female mice were sensitized subcutaneously into footpads with 100 μ g/50 μ l of KLH (Calbiochem) adsorbed on aluminum potassium sulfate (alum, Sigma-Aldrich), KLH alone or PBS alone. IL-1 α (100 ng/10 μ l PBS, Strathmann, Biotec AG) was injected into the same footpad on days 0, 1 and 2 of sensitization. On day 6, mice were challenged with 100 μ g/50 μ l KLH into the contralateral footpad. Footpad swelling was measured before and 24, 48 and 72 h after challenge. Six days later, popliteal lymph nodes were harvested and lymph node cells isolated by mechanical disruption. 1×10^6 lymph node cells were cultured in medium (500 ml RPMI-1640, 1% penicillin/streptomycin, 5% fetal calf serum, 1% essential amino acids, 1% glutamine, 1% 1 M HEPES) for 4 days. Cells were stimulated with 10 μ g/ml of KLH for antigen-specific cytokine release. Cytokine levels in supernatants were determined by ELISA.

Induction of Allergen-Induced Airway Disease

Mice were injected intraperitoneally with 20 μ g of OVA (chicken egg albumin, 5 $\times$ crystalline, Calbiochem) in 200 μ l alum on day 0 and boosted intraperitoneally at day 14. Challenge was performed by inhalation during days 27–29 with aerosols of OVA (1% solution in PBS), negative controls were nebulized with PBS alone. Murine recombinant IL-1 α (500 ng/200 μ l PBS/mouse) was administered intraperitoneally to different groups of OVA-sensitized BALB/c mice at the indicated time points.

Measurement of Airway Hyperresponsiveness

To assess airway hyperresponsiveness (AHR), we used an invasive whole-body plethysmograph as described previously [18]. Anesthetized mice were allowed to breathe spontaneously through intratracheal catheters (Mouse Ventilator, Ugo Basile). Calibrations and lung resistance measurements were performed by the Asthma Core Facility, Mainz, Germany, using a resistance/compliance isovolumetric analyzer according to the manufacturer's instructions (Buxco Electronics, Inc., BioSystem XA for Windows) [19]. On day 30, the mice were challenged with methacholine at rising concentrations (0–30 mg/ml). In order to determine AHR, the provocative concentration value was calculated for every individual mouse by linear interpolation of the dose-response curve as the methacholine dose causing 100% increase above baseline in lung resistance (PC100).

Bronchoalveolar Lavage Differential Cell Count and Cytokine Determination

After assessment of the AHR, bronchoalveolar lavage (BAL) was performed [20]. Mice were euthanized by an intraperitoneal injection of 5% ketamine-HCl (Ratiopharm) and 2% xylacin (Rompun®, Bayer) at a ratio of 1:1. The airways were flushed 5 times with 600 µl of PBS (total volume 3 ml/mouse). The lavage fluid was centrifuged for 10 min at 200 g and cell numbers were determined using trypan blue exclusion and a Neubauer chamber. A total of 8×10^4 cells were cytospun and air-dried. Differential cell counts were performed on DiffQuick (Dade Behring)-stained cytopsins of BAL cells using light microscopy ($\times 1,000$, oil immersion). The BAL fluid was centrifuged again at 200 g, 10 min at 4°C, and the supernatant was stored at -20°C pending cytokine analysis. IL-4, IL-5, IL-10, IL-13 and IFN- γ in BAL fluid were quantified using commercially available ELISA kits for IL-4, IL-10 (BD Biosciences) and IL-5, IL-13 and IFN- γ (R&D Systems).

Lung Histology

Lungs were collected 24 h after the last challenge and fixed in 10% formalin, dehydrated, mounted in paraffin, sectioned, and stained with PAS. PAS-positive epithelial cells were enumerated in 5–6 representative fields of each lung specimen using light microscopy ($\times 400$) and means calculated. Statistical analysis was performed with the mean value of each mouse.

OVA-Specific ELISA

After AHR determination, sera were collected and stored at -20°C pending determination of OVA-specific IgG2a and IgE by antigen capture ELISA. The antibody titer was defined by serum dilution of the samples. The IgE titer was calculated using linear regression analysis as the serum yielding an absorbance reading of OD = 0.2. Contents of IgG2a and IgE in serum samples were standardized using high-titer reference sera and expressed as units per milliliter.

Statistics

Statistical analysis was performed using the unpaired Student's *t* test. Differences in responsiveness to methacholine were assessed by repeated-measures ANOVA (dose response) and by one-way ANOVA (PC100). *p* values for significance were set at 0.05. Values for all measurements are expressed as means $\pm$ SEM.

Results

Effects of IL-1 α Administration on DTH

DTH reactions are primarily mediated by CD4+ T cells in which alum as adjuvant was shown to induce Th2-predominant antigen-specific immunity [15, 16]. To test the effect of IL-1 α administration during T cell priming on resulting immune responses, BALB/c mice were immunized using KLH with or without alum. In parallel, some mice were locally treated with 100 ng of IL-1 α after several hours and again on days 1 and 2 after sensitization. After 6 days, mice were challenged into the other

footpad by injection of KLH and footpad swelling responses were determined every 24 h (fig. 1a). As expected, DTH responses were stronger if mice were immunized in the presence of adjuvant as compared to KLH alone (right panel compared to middle panel). Unsensitized mice did not develop noticeable footpad swelling (left panel). Interestingly, in IL-1 α -treated mice, DTH in response to KLH and alum was significantly inhibited, i.e., 0.5 ± 0.1 versus 0.2 ± 0.2 mm⁻¹ after 48 h in PBS as compared to IL-1 α -treated mice (*p* $\leq$ 0.05).

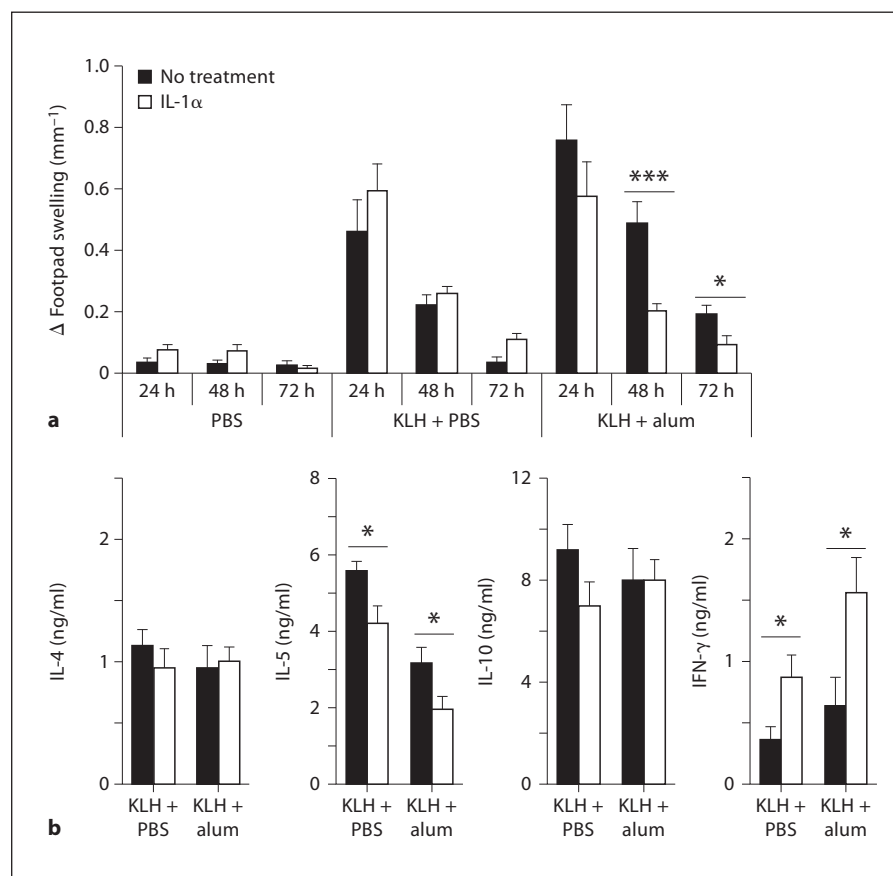
To determine the cytokine responses in KLH-immunized mice, lymph node cells were harvested 6 days after KLH challenge and restimulated in the presence (fig. 1b) or absence of KLH (data not shown). Antigen-specific release of Th2-associated cytokines was studied. The levels of IL-4 were unaltered by IL-1 α treatment, whereas release of IL-5 was diminished in mice after IL-1 α administration. In addition, IL-10 synthesis was not affected by IL-1 α . Interestingly, IL-1 α treatment during T cell priming induced significantly increased levels of IFN- γ from KLH/alum-sensitized lymph node cells (e.g. PBS vs. IL-1 α : 633 ± 242 vs. $1,560 \pm 287$ pg/ml/96 h, *p* $\leq$ 0.05). Thus, upon IL-1 treatment, the ratio between Th1/Th2 cells was shifted towards Th1.

IL-1 α Administration during T Cell Priming Prevents Induction of AHR

Next, we intended to study the effect of IL-1 α on the induction and maintenance of Th2-dependent murine AHR. After sensitization and a boost with OVA in the presence of alum (days 0 and 14), some mice were challenged by inhalation of OVA aerosols on 3 subsequent days (days 27–29; fig. 2a). Due to the fact that the timing of IL-1 α administration was critical for the induction of immunity in cutaneous leishmaniasis, 4 different treatment regimens were tested in the murine model of allergic asthma [3, 6]. IL-1 α (500 ng/ml) was delivered intraperitoneally (a) early (days 0–2), (b) during priming and boosting (days 0, 1 and 14), (c) during established Th2 immune responses (days 21–23) and (d) during the challenge performed with OVA inhalation (days 27–29; fig. 2a).

On day 30, airway resistance after methacholine challenge in each treatment group was assessed by invasive body plethysmography (fig. 2b) [18, 19]. Baseline resistance values did not differ between the different groups. As compared to PBS sensitization, OVA sensitization dramatically increased the airway resistance after methacholine treatment by about 300% of baseline as expected. If the mice had been treated with IL-1 α only during sen-

Fig. 1. Decreased footpad swelling during DTH responses in BALB/c mice treated with IL-1 α during sensitization. **a** 3–5 BALB/c mice per group were sensitized on day 0 with KLH/alum or antigen (KLH + PBS) or PBS alone as controls into their footpads. IL-1 α (100 ng/mouse) was injected on days 0–2. One week later, mice were challenged with KLH into their contralateral footpads and footpad swelling was assessed (mean $\pm$ SEM, 3 independent experiments, $n \geq 12$; * $p \leq 0.05$, *** $p \leq 0.002$). **b** Six days after challenge with KLH, popliteal lymph nodes were collected for antigen-specific cytokine assessment. Supernatants were assayed for the presence of IL-4, IL-5, IL-10 and IFN- γ by ELISA (means $\pm$ SEM, 3 independent experiments, $n \geq 10$; * $p \leq 0.05$).



sitization (days 0–2) or on days 0, 1 and 14, the resulting airway resistance was significantly improved as compared to PBS-treated control mice (231 ± 16 vs. $286 \pm 13\%$ for the group treated with IL-1 α on days 0, 1 and 14 as compared to the control treatment, $p \leq 0.05$; fig. 2b, left panel). In contrast, if IL-1 α was delivered at later time points (days 21–23 and days 27–29), an even higher airway resistance was observed (fig. 2b, right panel).

In addition, we calculated PC100 values for each of the treatment groups. Statistically significant differences were found for the groups treated with IL-1 α on days 0–3 and 0, 1 and 14 (17.8 ± 2.5 and 16.3 ± 2.3 mg/ml, respectively) as compared to PBS-treated OVA-sensitized mice (11.5 ± 1.7 mg/ml, $p \leq 0.05$). In contrast, the PC100 values for IL-1 α treatment on days 21–23 and 27–29 were significantly lower than in our positive control (IL-1 α days 21–23: 4.9 ± 1.0 mg/ml, days 27–29: 5 ± 1.4 mg/ml, $p \leq 0.05$). Untreated controls showed a PC100 value of 23.9 ± 1.4 mg/ml.

After assessments of airway resistance, the mice were sacrificed, the lungs harvested and histologically investi-

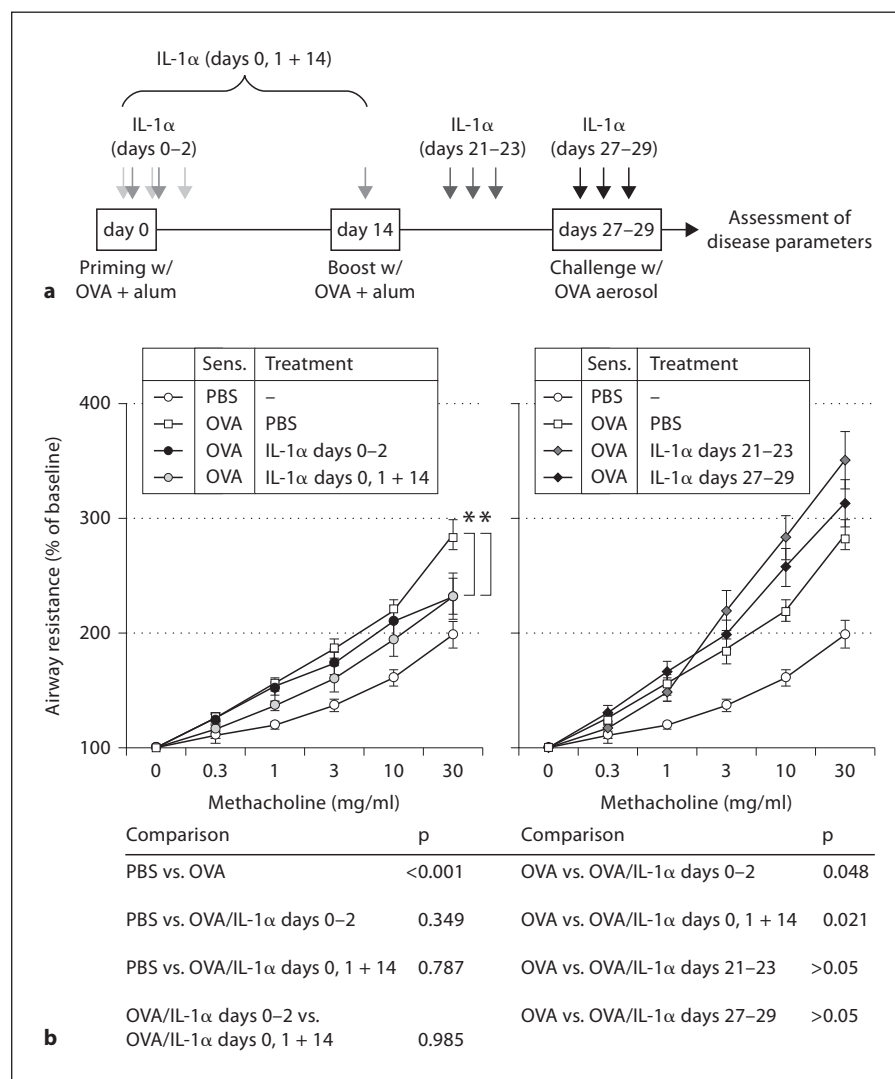
gated for the number of bronchi infiltrated with eosinophils (fig. 3a). As expected, lung infiltration with eosinophils generally correlated with increased mucus production and goblet cell hyperplasia as depicted in the representative pictures. In parallel to the percentage of infiltrated bronchi, the number of PAS-positive epithelial cells was significantly smaller in mice receiving IL-1 α at days 0–2 and days 0, 1 and 14 (fig. 3b), whereas later treatment with IL-1 α did not show any effect. Thus, the degree of airway resistance correlated with lung inflammation under these conditions.

In summary, similarly to the model of murine cutaneous leishmaniasis, the timing of IL-1 α administration was critical in allergic asthma: early IL-1 administration improved disease outcome, whereas late delivery did not.

Decreased Eosinophilia, IL-5 and IgE Levels in the BAL and Serum of IL-1 α -Treated Mice

Next, BAL fluid was obtained after OVA aerosol challenge of IL-1 α -treated and control mice on day 30 [20].

Fig. 2. Early administration of IL-1 α inhibits AHR in experimental allergen-induced airway disease. **a, b** Groups of ≥ 3 BALB/c mice were sensitized with 100 μ g of OVA/alum or PBS and boosted on day 14. Some mice were treated intraperitoneally with IL-1 α (500 ng/mouse) at different time points during and after sensitization. On days 27–29, mice were repeatedly challenged with OVA aerosols. **b** 24 h later, AHR responses to methacholine were measured by invasive plethysmography (means $\pm$ SEM; data are pooled from 5 independent experiments for each experimental group, $n \geq 20$ mice; * $p \leq 0.05$). Statistical comparisons between treatment groups were performed using the repeated-measures ANOVA.



Both the cellular composition (fig. 4) and the cytokine level in the BAL (fig. 5) were determined. In addition, we determined the levels of OVA-specific antibodies in the serum of sensitized and challenged mice on day 30 (fig. 6).

Upon sensitization with OVA and alum, the number of cells/BAL increased about 3-fold to $2.9 \pm 0.5 \times 10^6$ /mouse as compared to negative controls ($1.1 \pm 0.4 \times 10^6$). No significant difference was found in the number of cells obtained from the various IL-1 α treatment groups as compared to control treatment (data not shown). After determination of the total cell number obtained from each mouse, the cells were cytopun and stained using DiffQuick. Interestingly, the percentage of eosinophils in the BAL fluid significantly decreased in mice treated

with IL-1 α on days 0–2 or on days 0, 1 and 14 as compared to OVA-sensitized positive controls [$31.6 \pm 1.4\%$ ($9.4 \pm 2.7 \times 10^5$ cells/BAL) and $23.6 \pm 1.5\%$ ($8.7 \pm 1.4 \times 10^5$) vs. $39.5 \pm 2.1\%$ ($13.1 \pm 2.8 \times 10^5$), $p < 0.005$ and $p < 0.002$, respectively]. Interestingly, the number of neutrophils in the BAL inversely correlated with the eosinophil count, i.e., in both treatment groups receiving early IL-1 α the number of neutrophils increased from $2.1 \pm 0.4\%$ ($0.6 \pm 0.1 \times 10^5$ cells/BAL) to $8.1 \pm 1\%$ ($3.1 \pm 0.8 \times 10^5$) and $9.4 \pm 1.5\%$ ($3.3 \pm 1 \times 10^5$), respectively. In contrast, if IL-1 α was administered when Th immunity was already established (days 21–23 and days 27–29) no dramatic alterations of the cellular infiltrate were observed, which was consistent with the findings in AHR. Differences in the numbers of lymphocytes and macrophages

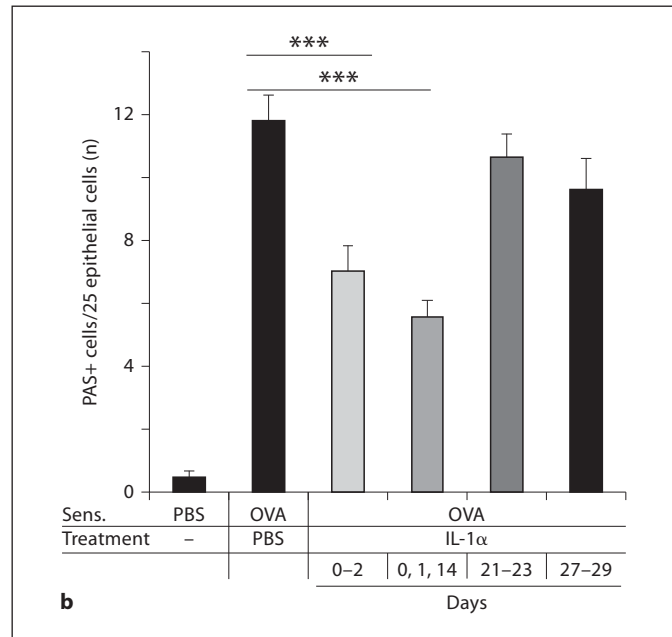
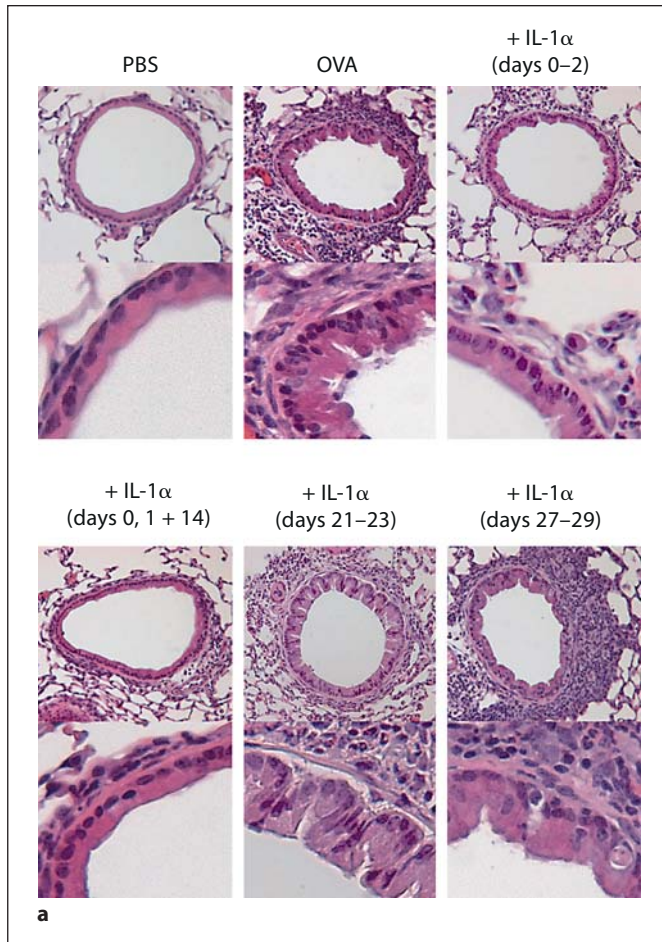


Fig. 3. Decreased inflammatory lung infiltration and mucus production in mice treated with IL-1 α during sensitization. Lungs were collected on day 30, embedded in paraffin and sections were stained with HE (**a**) or PAS (**b**). **a** Representative histological images are shown (upper panel: $\times 400$, lower panel: $\times 1,000$). **b** In representative microscopic fields, the number of PAS-positive cells among 25 cells was determined. Pooled data from 5 independent experiments/experimental group are shown as means $\pm$ SEM, $n \geq 25$ mice per treatment group; *** $p \leq 0.002$).

in the BAL fluid were not detected upon IL-1 α treatment.

As expected, in OVA-sensitized mice, the amounts of Th2-derived IL-4 (data not shown), IL-5, IL-13 and IL-17A were increased after aerosol challenge (fig. 5). Early treatment with IL-1 α during T cell priming resulted in a significantly reduced production of IL-5, a cytokine that is responsible of eosinophil recruitment into inflamed lungs [17], as detected in the BAL fluid (82 ± 19 and 81 ± 16 as compared to 205 ± 33 pg/ml in control mice, $p \leq 0.002$ and $p \leq 0.005$). In parallel, IL-13 and IL-17A synthesis was also diminished in these groups, although the data did not reach statistical significance. In contrast, late IL-1 α administration (days 21–23) increased the amount of IL-13 found in the BAL without affecting the already maximal IL-5 release observed in positive controls. Other cytokines were not significantly altered (IL-10 and IL-4) or detectable (IFN- γ) upon IL-1 α treatment (data not shown).

Finally, the levels of Th2-associated OVA-specific IgE and IgG1 antibodies as well as Th1-associated IgG2a were determined in the serum of asthmatic mice. OVA sensitization of BALB/c mice induced dramatically elevated levels of IgE, IgG1 and IgG2a. In parallel to the decreased airway resistance in mice treated with IL-1 α early on, we detected significantly lower levels of OVA-specific IgE and IgG1 in these treatment groups (fig. 6). In contrast, the levels of IgG2a associated with Th1 immunity were increased upon early IL-1 α treatment. Other treatment regimens at later time points using IL-1 α did not alter the antibody response.

In summary, upon early treatment with IL-1 α , OVA-induced AHR, peribronchial infiltrates, BAL eosinophil counts, IL-5 and IgE levels were decreased. In contrast, disease parameters showed the opposite or no change if IL-1 was administered at the time of allergen challenge.

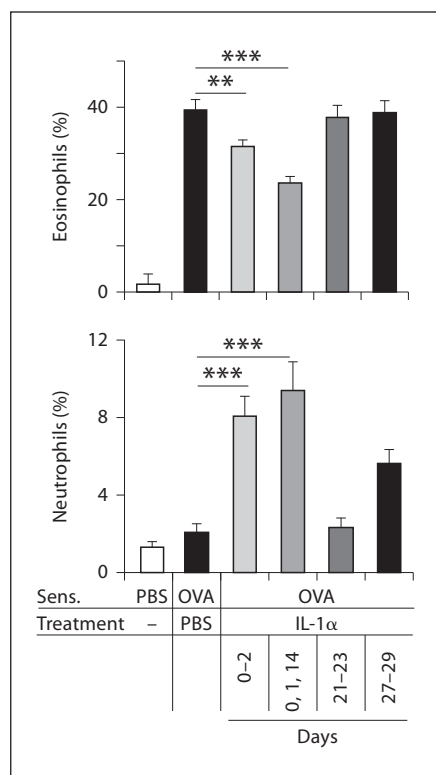


Fig. 4. IL-1 α treatment decreases eosinophil immigration into inflamed lungs. IL-1 α (100 ng/mouse) was injected at early time points during sensitization on days 0–2 or 0, 1 and 14 or at later time points (days 21–23 or 27–29). After challenge, the BAL fluid was extracted. Total BAL cell numbers were determined. The composition of the BAL infiltrate was determined on cytopins (mean $\pm$ SEM of 13 independent experiments, $n \geq 29$ mice in every treatment group; ** $p \leq 0.005$, *** $p \leq 0.002$ as compared to positive controls).

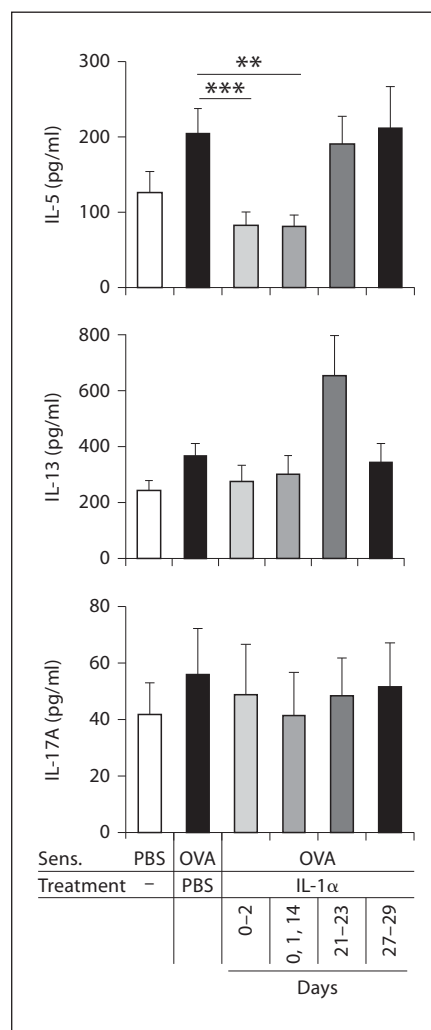


Fig. 5. Increased Th2 cytokine levels in AHR reactions after IL-1 α treatment during sensitization. BALB/c mice were sensitized as described above. rIL-1 α was administered during sensitization or at later time points as indicated. After challenge, the BAL fluid was extracted. The BAL fluid cytokine levels were determined by ELISA (means $\pm$ SEM, 5 independent experiments/experimental group, $n \geq 32$ mice; ** $p \leq 0.005$, *** $p \leq 0.002$ as compared to positive controls sensitized with OVA alone).

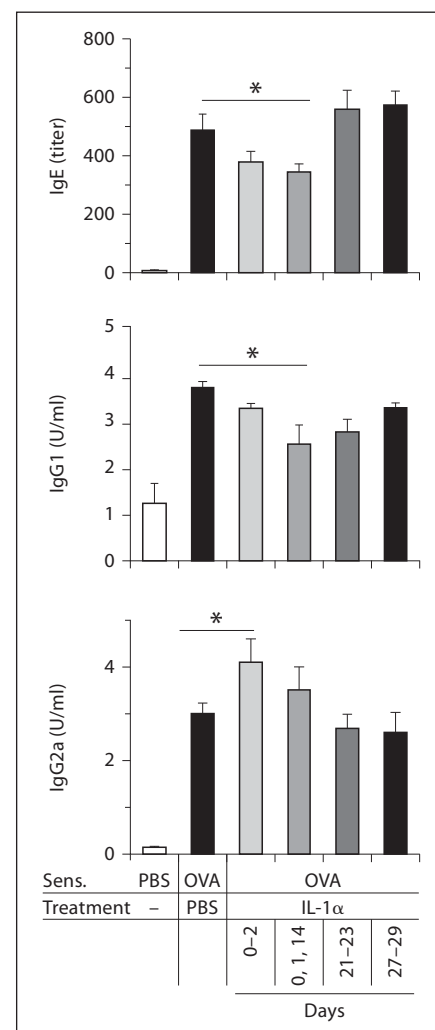


Fig. 6. IL-1 α administration during OVA sensitization decreases IgE and IgG1 production. The BAL fluid of BALB/c mice sensitized and challenged with OVA was extracted. Intraperitoneal treatment with IL-1 α was performed on the days indicated. The levels of OVA-specific serum immunoglobulins were determined by ELISA (3 independent experiments/experimental group, * $p \leq 0.05$, $n \geq 15$ mice/group).

Discussion

Twin studies showed a heritability of asthma of about ~60% [21], and several studies suggested that the IL-1 gene complex is involved in the regulation of IgE-mediated

atopic reactions [22–26]. Interestingly and supportive for a role for IL-1 in allergic asthma, IL-1 β serum and BAL levels as well as submucosal IL-1 immunostaining were increased in asthmatic patients [27–29]. In this study, we demonstrated that the effect of IL-1 in experi-

mental allergic responses is critically dependent on the time of treatment: if sufficient amounts of IL-1 α were present during allergen sensitization, preferential Th1 immunity was observed with alleviated disease whereas at later time points, IL-1 administration was without significant effect on the Th2-mediated allergic response.

Prior studies by several laboratories including ours have demonstrated that IL-1 α / β -mediated effects on different Th subpopulations depend on the IL-1RI expression levels by Th cells [2, 3, 5, 30, 31]. Early after infection with *L. major*, IL-1-mediated protection was dependent on naïve T cells expressing IL-1RI, whereas high levels of IL-1RI on established Th2 cells at later time points after infection promoted Th2 expansion, correlating with disease progression [3, 6]. Thus, using experimental leishmaniasis as a disease model for the regulation of Th1/Th2 immunity, we identified an important role for IL-1 in the decision between disease resistance and susceptibility. In the present study, using a murine model of allergic asthma, we applied these findings in another highly relevant Th2 disease model. Interestingly, similar time-dependent effects of IL-1 α on Th1/Th2 priming were observed in this model of a common human disease.

Our findings are only partially supported by described IL-1 effects in murine asthma. Previous reports showed that IL-1 signaling promotes asthma development. To study this, mice deficient in the IL-1RI $-/-$, IL-1 receptor antagonist (IL-1RA) $-/-$, IL-1 α / β $-/-$ and depletion experiments were used. Two different lines of IL-1RI $-/-$ mice have been generated independently: One IL-1RI $-/-$ BALB/c strain [32] exhibited no phenotype in severe OVA/alum-induced asthma. In contrast, in a model of a more mild asthma induced without adjuvant, these mice showed weaker AHR with significantly lower IgE levels, less lung infiltration with eosinophils and goblet cell hyperplasia [33]. The other IL-1RI $-/-$ mouse line on a mixed C57BL/6 $\times$ 129 background [34] showed reduced eosinophil recruitment into lungs due to decreased expression of VCAM-1 during antigen-induced AHR [35] and improved disease outcome [29]. In other studies, administration of anti-IL-1 α /anti-IL-1 β during sensitization and challenge abrogated the development of asthma [29]. In addition, IL-1 α / β $-/-$ mice showed significantly reduced asthma development, whereas responses seen in IL-1RA $-/-$ mice were severely exacerbated [36]. Taken together, these studies suggest that total absence of IL-1 signaling leads to abrogation of AHR in allergic asthma. In contrast to the findings in genetically modified mice and antibody depletion, we now report that IL-1 exerts effects on allergic asthma that are strictly dependent on the time of treat-

ment. Using genetically modified mice, these dual effects cannot be easily revealed. In addition, the effect of IL-1 during sensitization only has not been studied previously.

In some reports, the effect of IL-1/IL-1RI only during allergen challenge was studied. Here, IL-1 primarily promoted eosinophil recruitment and thereby worsened disease outcome. IL-1 α caused severe pulmonary infiltration with neutrophils/macrophages and emphysema development in transgenic mice with timed induction of IL-1 α expression in lung epithelial cells [37]. In addition, delivery of an adenovirus expressing IL-1RA after sensitization and before airway antigen challenge alleviated AHR, pulmonary infiltration with eosinophils and neutrophils and decreased IL-5 levels [38], IL-1RA abrogated both the IL-5- and IgE-induced changes in airway smooth muscle responsiveness in asthma [39], whereas, in contrast, IL-1 β and TNF- α increased airway smooth muscle response to various contractile agonists, e.g. methacholine [40]. Finally, IL-1 β and TNF- α induce NF- κ B-dependent CCL28 induction, a mucosal chemokine that attracts eosinophils and T cells via CCR3 and CCR10 [41]. Thus, the latter findings are in line with our observation that IL-1 α administered to mice just before or during allergen challenge (days 21–23 and days 27–29) worsened disease outcome most likely due to increased eosinophil recruitment and smooth muscle responsiveness, and blockade of IL-1 (e.g. by IL-1RA administration) reduces disease severity.

The cells involved in the induction of allergic responses are DCs, mast cells, T and B cells [42]. Developing allergen-specific Th2 cells secrete cytokines such as IL-4, IL-5, IL-13, and induce allergen-specific IgE and IgG1, all of which are responsible for resulting increased airway resistance upon re-challenge with antigen. DCs play important roles not only in bridging innate and adaptive immunity by presenting antigen to naïve T cells but also by directing resulting immune responses via release of certain cytokines/chemokines. In murine asthma, DCs appear to play a role both as one of the major APCs during sensitization, but they also accumulate in the lung afterwards and promote airway inflammation after re-exposure to allergen [43–46]. The question as to which cells may contribute to IL-1 release in allergic asthma is still unanswered. Most likely, DCs (similar to murine leishmaniasis [2, 3]) and macrophages are both candidates. Future studies using targeted expression of IL-1 by these cells will aid to solve this question.

Erb et al. [47] previously demonstrated that intranasal mycobacterial infection suppressed the development of OVA-induced asthma. Intranasal *Mycobacterium bovis*-

bacillus Calmette-Guérin infection before allergen challenge resulted in decreased eosinophilia and AHR, decreased IL-5 levels and elevated IFN- γ release. Prior studies showed that infection with *Mycobacteria* leads to strong release of IL-1 from DCs and macrophages and the absence of IL-1R signaling is associated with a dramatic defect in early control of mycobacterial infection [48]. Thus, the suppressive effect of mycobacterial infection on allergic asthma may also be caused by release of IL-1.

The role of the Th1/Th2 paradigm in DTH is less clear. Both Th1 and Th2 immunity were observed after immunization with different adjuvants [15, 16]. Sensitization with KLH (plus alum) induced strong Th2-mediated humoral responses [49, 50]. Using this Th2-type model disease, we observed that IL-1 administration during sensitization abrogated disease and preferentially induced IFN- γ as compared to Th2-associated IL-5. In parallel, studies using IL-1RI $^{-/-}$ mice or adoptive transfer experiments with IL-1 α/β $^{-/-}$ and IL-1RA $^{-/-}$ cells showed that IL-1 β was necessary for efficient Th1 priming in DTH

[51, 52]. In addition, CD4 $^{+}$ T cell-derived IL-1 α/β was also required for the induction of TNF- α from DCs, which in turn activated allergen-specific T cells. Thus, similar to our findings, a Th1-immunomodulatory role of IL-1 in DTH responses was revealed.

In summary, the present study demonstrates an important role of IL-1 in the regulation of Th development in Th2-mediated allergic disease. Future studies will be directed at characterizing the alterations in allergic asthma mediated by IL-1 in more detail. These studies will improve our understanding of the exact mechanisms by which IL-1 regulates Th differentiation in this important model of a human disease.

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