



Endogenous IL-18 in experimentally induced asthma affects cytokine serum levels but is irrelevant for clinical symptoms

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Abstract

T cells and T cell derived cytokines are involved in the complex pathogenesis of asthma. The role of the cytokine IL-18 however, is not clearly defined so far. On the one hand side IL-18 induces Th1-type cytokines and thereby might counter-regulate Th2-mediated allergic asthma. On the other hand IL-18 also bears pro-inflammatory effects possibly enhancing experimental asthma. In order to elucidate the role of IL-18 in allergic pulmonary inflammation typical symptoms were compared after induction of experimental asthma in IL-18^{-/-} and in wild type mice. Asthma was induced using ovalbumin (OVA) as allergen for sensitization and challenge. Sham sensitized and OVA challenged mice served as controls. Bronchoalveolar lavage-fluid cytology, leukocyte infiltration in lung tissues, serum levels of OVA-specific IgE and cytokines, and lung function were analyzed. Clear differences could be observed between control and asthmatic mice, both in wild type and IL-18^{-/-} animals. Surprisingly, no differences were found between asthmatic wild type and IL-18^{-/-} mice. Thus, in contrast to conflicting data in the literature IL-18 did not suppress or enhance the pulmonary allergic immune response in a murine experimental model of asthma.

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1. Introduction

The balance of pro- and anti-inflammatory cytokines plays a central role in the outcome (healing or chronic stage) of an inflammatory reaction. The cytokine IL-18, formerly termed interferon- γ inducing factor (IGIF), favors a pro-inflammatory Th-1 type immune response by enhancing the secretion of pro-inflammatory mediators such as TNF- α , IL-8, and IFN- γ . Thus, IL-18, which belongs to the IL-1 cytokine family, constitutes an important factor within the inflammatory reaction. It strongly resembles

IL-1 β in structure but its binding to target cells is mediated by a distinct receptor complex [1]. Upon formation of the active receptor complexes, IL-1 β and IL-18 use common signaling mechanisms, for instance the recruitment of the initial MyD88/IRAK/Traf6 signaling complex, finally leading to nuclear translocation of transcription factors, e.g. NF- κ B. It is well documented that IL-18 as well as IL-1 β is processed primarily by caspase-1, however, alternative pathways of processing of IL-18 are known [2]. Candidates for the transformation of inactive proIL-18 into the biologically active cytokine are intracellular proteases e.g. MMP-9, Proteinase-3, and mast cell-chymase. Consequently, the inhibition of caspase-1 in order to block IL-18 activity offers only limited options for therapeutic interventions. Another therapeutic approach considers the IL-18 binding protein

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(IL-18BP). IL-18BP has a high affinity to IL-18 and represents an antagonistic activity in the regulation of IL-18 activity. The balance between IL-18 and IL-18BP, which finally defines the net IL-18 bioactivity, is affected by IFN- γ (among others), which promotes the release of IL-18BP via a negative feedback loop. Deregulated IL-18 expression is associated with a number of chronic diseases such as Crohn's disease, insulin-dependent diabetes mellitus, atherosclerosis, and inflammatory arthritis [3]. Furthermore, IL-18 seems to be involved in the development of bronchial asthma, since patients with a long history of asthma show increased serum levels of IL-18 [4,5]. In addition, mice overexpressing IL-18 or mice treated daily for a period of 2 weeks with exogenous IL-18 spontaneously develop high serum levels of IgE, a typical symptom of allergic asthma. However, although induction of this IgE response was dependent on IL-4 and Stat6, a Th2 polarization in these mice, defined by a high IL-4/IFN- γ ratio, was undetectable [6,7].

In experimental mouse models of lung disease, the role of IL-18 is still discussed controversially. When applied intranasally to normal mice, IL-18 in combination with IL-2 induces airway hyperresponsiveness (AHR), mucus hypersecretion, and CD4 T cell-dependent infiltration of eosinophils in the lung [8], while its intraperitoneal (i.p.) application resulted in an interstitial infiltration of mainly NK cells with lethal outcome [9]. Moreover, injection of IL-18 in normal mice induced the secretion of IgE in a CD4 T cell- and IL-4-dependent manner [6]. In a model of cockroach antigen-induced airway allergy, systemic neutralization of IL-18 by administration of an anti-IL-18 antibody accelerated but did not augment the peribronchial influx of eosinophils. In contrast, IL-18 applied locally together with the challenging antigen increased the total number of eosinophils in peribronchial spaces and BAL-fluid but did not alter the kinetic of extravasations [10]. Similar results were found in mice which have been sensitized by adoptive transfer of OVA-specific Th1-type, but not Th2-type, T cells and challenged with OVA plus IL-18. In such experimental setting, airway responsiveness and the number of eosinophils in BAL-fluid were enhanced by the addition of IL-18 in comparison to sole OVA inhalation. Thus, experimental allergic airway disease seems to be based not only on Th2-type but also on Th1-type mechanisms [11]. Finally, it was shown that i.p. administration of IL-12 plus IL-18, but not IL-18 alone, 30 min before initiation of the challenge procedure in an OVA-induced allergic asthma model reduced asthma-like symptoms, concomitant with an inhibition of allergen-specific Th2-like development [12].

In the present study we used IL-18-deficient C57BL/6 mice in the OVA-induced asthma model in order to elucidate a role for IL-18 in this allergic response. We demonstrate that the absence of IL-18 does not affect any of the asthma-specific parameters analyzed, allergen-specific serum IgE titer, histological derangements of the lung, number of infiltrating leukocytes, serum cytokine levels, and AHR.

2. Material and methods

2.1. Animals

Female C57BL/6 mice ('wt' Charles River, Sulzfeld, Germany) and B6.129P2-*Il18*^{tm1Aki}/J ('IL-18^{-/-}', kind gift of S. Akira, Osaka, Japan) were used at an age of 6–8 weeks throughout the study. Mice were fed with ovalbumin-free laboratory food and tap water *ad libitum* and held in a regular 12 h dark/light cycle at a temperature of 21 ± 2 °C and 50% humidity under specified pathogen free conditions. All animal manipulations were performed in accordance with the German animal protection law under a protocol approved by the appropriate governmental authority (Bezirksregierung Hannover).

2.2. Sensitization and allergen challenge of mice

Experimental asthma was induced by sensitization with ovalbumin (OVA, Sigma, Taufkirchen, Germany) Grade VI and challenging with OVA Grade V. For sensitization mice were injected i.p. on day 1, 14, and 21 with 10 µg OVA absorbed to 1.5 mg aluminium hydroxide (alum, Pierce Biotechnology, Rockford) solubilized in phosphate buffered sodium chloride ('asthma-like'). Control animals were sham sensitized with 1.5 mg alum in PBS only. On day 28 and 29 all mice were challenged by 20 min exposition to 1% OVA/PBS in a nebulizer box, in which mice could move freely. No sickness or restrictions could be noticed in experiments according to this protocol. Four experimental groups are at the basis of this study: (1) wt asthma-like, (2) wt control, (3) IL-18^{-/-} asthma-like, and (4) IL-18^{-/-} control were examined. All groups included at least 20 mice; however, not all assays were performed including all mice.

2.3. Measurement of lung function

Twenty-four hours after the last allergen challenge, airway responsiveness to inhaled methacholine was assessed. Pulmonary function was measured non-invasively with a glass-made head-out body plethysmograph system for four mice as previously described. Briefly, the conscious, spontaneously breathing mice were placed in the body plethysmographs while the head of each animal protruded through a neck collar into a ventilated head exposure chamber. Tidal midexpiratory flow (EF₅₀) was calculated from the pneumotachograph flow signal for each breath (HEM 3.5, Notocord). EF₅₀ (ml/s) is defined as the tidal flow at the midpoint (50%) of expiratory tidal volume and has been established as a measure of bronchoconstriction [13,14].

2.4. Animal dissection

After testing the lung function mice were killed with an i.p. overdose of a mixture of rompun (Bayer Vital GmbH,

Leverkusen, Germany) and ketamin (A. Albrecht GmbH, Aulendorf, Germany). Mice were bled by heart puncture and sera were prepared and frozen at -80°C . The lungs were lavaged individually (bronchoalveolar lavages: BAL) in situ with a 1 ml aliquot of sterile PBS. Mediastinal lymph nodes were prepared and shock frozen for subsequent RNA extraction. Lungs were either fixed in 4% formalin via the trachea using 15 cm water columns and embedded in paraffin or were shock frozen in liquid nitrogen for subsequent RNA extraction or homogenization. Homogenization was performed mechanically in 1 ml PBS containing $1\times$ protease inhibitors (Complete protease inhibitor cocktail, Roche, Mannheim, Germany) using an electric device. Cellular debris were pelleted by centrifugation (15,000g, 15 min, 4°C) and the cleared supernatants were analyzed for total protein (Bradford protein assay, BioRad, Hercules, CA, USA) and cytokine concentrations.

2.5. Differential cell count

Total cell counts in BAL-fluid were scored using a Zeiss (Telaval 31) microscope and viability was determined by trypan blue dye exclusion using a Neubauer chamber. Leukocyte subsets (eosinophils, neutrophils, macrophages, or lymphocytes) were enumerated in BAL-fluid using cytopins (centrifuged preparations) stained with Diff-Quik (Medion Diagnostics GmbH, Dueningen, Germany). A total of 400 cells of each sample were counted.

2.6. Histology

Fixed and paraffin embedded lungs were cut into $4\text{ }\mu\text{m}$ slices and stained with hematoxylin/eosin. From each lung six sections (containing hilar-structures and periphery) of both the right and left lobes were evaluated. The intensity of bronchial inflammation was measured by counting cell nuclei within grids. In four different alveolar sections of one slide per animal numbers of nuclei were determined. The nuclei within an area which was adjacent to the basal membrane of two different vessels and bronchi have been counted as well to estimate the cellular perivascular or peri-bronchial infiltration. Microphotographs were performed using a Nikon Eclipse 50i microscope with a Nikon Digital Sight DS-U1 Camera.

2.7. Evaluation of cytokine concentrations

The cytokine levels in sera were measured using the FlowCytomix Multiplex Kit (Th1/Th2 10plex; Bender-MedSystems, Vienna, Austria). Ten cytokines, i.e. GM-CSF, IL-1 α , IL-2, IL-4, IL-5, IL-6, IL-10, IL-17, IFN- γ , and TNF- α were detected simultaneously. The assay was performed according to the instructions supplied by the manufacturer. In lung homogenates and BAL-fluids IL-5, IL-10, IL-12, IL-13 (R&D Systems, Minneapolis, MA, USA), and eotaxin (RayBiotech, Norcross, GA, USA)

levels were analyzed using commercially available ELISA tests accordingly to the manufacturers' instructions.

2.8. Mouse anti-OVA IgE ELISA

Serum levels of OVA-specific IgE were analyzed by ELISA, using a mouse-specific anti-IgE-antibody. Briefly, OVA coated microtiter plates (Nunc, Roskilde, Denmark) were incubated with test sera, diluted 1:200 in PBS containing 5% BSA, and serial dilutions of a standard serum obtained from a hyperimmunized mouse. Mouse IgE was detected by subsequent incubation with a biotinylated rat anti-mouse IgE antibody (BD/Pharmingen, Franklin Lakes, NJ, USA) and peroxidase-coupled streptavidine (Biosource). Peroxidase-dependent conversion of TMB was quantified by reading at 405 nm. Titers were calculated according to the standard serum obtained from a hyperimmunized mouse, which was set at 1×10^6 IU/ml.

2.9. RNA extraction and quantitative RT-PCR

RNA was extracted from lymph nodes and lung tissue using the Trizol method (Invitrogen, Carlsbad, CA, USA). Homogenization and lysis of solid tissue was performed in lysis buffer by repeated passing through a series of syringes with successively decreasing diameters. Total RNA was purified essentially as described by the manufacturer. Total RNA was reverse transcribed into cDNA (QuantiTect RT Kit, Qiagen, Hilden, Germany) and quantitative real-time PCR (SYBR Green PCR Kit, Qiagen) was performed using the following primers: IL-18 sense: 5'-AAT GAC CAA GTT CTC TTC GTT GAC-3', IL-18 antisense: 5'-ACA GCC AGT CCT CTT ACT TCA C-3', IL-18R α sense: 5'-TGT GCT TGT TCT CGC CTC TG-3', IL-18R α antisense: 5'-ACC ATC TGT TAG TGT CTC GTC TC-3'.

2.10. Statistical analysis

Cytokine levels in lung homogenates were analyzed after normalizing the cytokine concentrations of individual homogenates according to the corresponding protein concentrations. Statistical analyses were performed by calculating means $\pm$ SEM for each parameter within an experimental group. Analysis of significance was performed using Mann-Whitney-U-test. *p*-Values <0.05 (*) and <0.01 (**) were considered significant.

3. Results

3.1. IL-18 and IL-18 receptor expression in asthmatic C57BL/6 mice

Induction of asthma is accompanied by a preferential development of naïve T cells towards the Th2-type. IL-18 is a cytokine with primarily Th1 related function, it however can enhance also Th2 prototypic IL-4 expression. In

homogenates of lungs from asthmatic mice an enhanced level of IL-18 is observed in comparison to controls (Fig. 1). In addition the mRNA expression of IL-18 receptor- α in lungs, which is indicative for IL-18 activity [15], and of IL-18 in lung draining lymph nodes is higher in OVA-sensitized and challenged mice in comparison to controls (not shown). These observations point to a contribution of IL-18 to the allergic reaction, justifying the analysis of induction of experimental asthma in the absence of endogenous IL-18.

3.2. Antigen-induced bronchial infiltration is not affected by deletion of the *il18* gene

A robust infiltration of eosinophils in the lungs is a central symptom of allergic asthma, detectable e.g. by differential cell count in BAL-fluid (BALf). In our experimental setting, the absolute number of lung-infiltrating cells is enhanced in asthmatic ($1.54 \pm 0.69 \times 10^6$ cells/mouse) in comparison to control mice ($0.25 \pm 0.11 \times 10^6$ cells/mouse). In IL-18 $^{-/-}$ mice the absolute numbers of infiltrating cells are found to be comparable to those found in wt mice (asthma: $1.48 \pm 0.80 \times 10^6$ cells/mouse; control: $0.31 \pm 0.17 \times 10^6$ cells/mouse). Differential cell counts reveal that the majority of infiltrating cells in control mice, both wt and IL-18 $^{-/-}$, consist mainly of alveolar macrophages and of some lymphocytes, while eosinophils are virtually absent. In contrast, after induction of experimental asthma both strains display significantly higher numbers of eosinophils, lymphocytes, and macrophages in bronchial infiltrates (Fig. 2). Thus, in terms of cellular infiltrations in the lungs of asthmatic mice no significant differences between wt and IL-18 $^{-/-}$ mice are detectable (Fig. 2). In comparison to untreated mice, sham sensitization and OVA challenge does not affect the morphological appearance of lungs of neither wt nor of IL-18 $^{-/-}$ mice (not

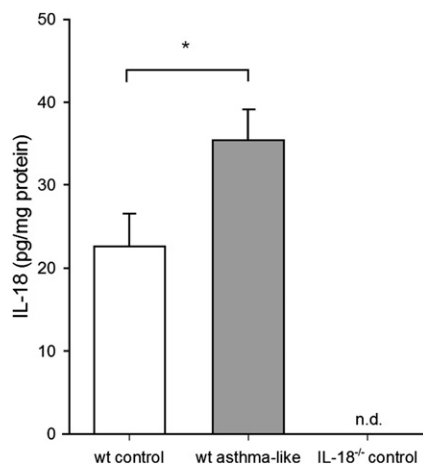


Fig. 1. IL-18 levels in lungs of wt mice are enhanced due to OVA sensitization and challenge. IL-18 concentrations were measured in homogenates of lungs of asthmatic ($n = 8$) and control ($n = 5$) mice by ELISA. Reported are mean IL-18/total protein values $\pm$ SEM (n.d., not detectable; * $p < 0.05$, ** $p < 0.01$).

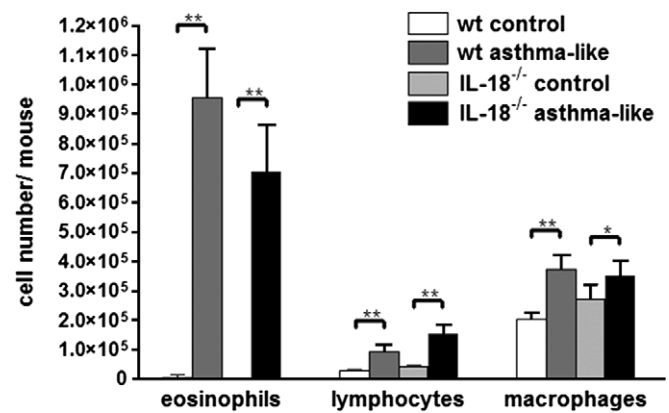


Fig. 2. Experimental asthma leads to enhanced numbers of eosinophils, lymphocytes, and macrophages in BALf of both, IL-18 $^{-/-}$ and wt mice. Cells present in bronchoalveolar spaces were collected by bronchoalveolar lavage. Cell numbers were evaluated after cytopsin of the BALf and subsequent Diff-Quik staining. Shown are mean numbers $\pm$ SEM ($n = 15$ /group) of the cell populations indicated on the x-axis within BALf obtained from the four experimental groups (* $p < 0.05$, ** $p < 0.01$).

shown). In the asthmatic mice dramatic infiltration of inflammatory cells is observed also by histological analysis, however, in both strains it appears to be very similar (Fig. 3A). Applying a quantitative evaluation of the lung sections shown in Fig. 3A, it is found that the allergen-induced infiltrating cells accumulated in peribronchial and perivascular regions, while they do not appear in the alveolar spaces (Fig. 3B).

3.3. Deletion of IL-18 affects antigen-induced serum IL-5 and IL-17 expression

Sensitizing and challenging mice with OVA results in the appearance of systemic OVA-specific IgE. Accordingly, OVA-specific IgE is virtually absent in sera of both, wt and IL-18 $^{-/-}$ control mice, while it is detected in large quantities in sera of asthmatic mice, however, without differences between wt and IL-18 $^{-/-}$ mice (Fig. 4).

In addition to allergen-specific IgE, sera of asthmatic mice show a bias of cytokines towards a Th2-type profile. Accordingly, we find enhanced serum IL-5 levels due to induction of experimental asthma in both, wt and IL-18 $^{-/-}$ mice. While a statistically significant difference can be scored between mean IL-5 levels of control and asthmatic wt mice (36.9 ± 24.3 pg/ml and 239.0 ± 54.9 pg/ml, respectively), mean levels of asthmatic wt and asthmatic IL-18 $^{-/-}$ (114.1 ± 56.5 pg/ml) mice do not differ significantly. However, taking into account the number of animals with IL-5 serum levels above detection limit, a considerable difference between asthmatic wt (11/15) and asthmatic IL-18 $^{-/-}$ (5/15) mice appears (Fig. 5, upper panel). Thus, endogenous IL-18 seems to have a limited effect on the expression of IL-5 accompanying induction of experimental asthma in C57BL/6.

IL-4 and TNF α are undetectable, while the serum concentrations of IL-12p70 and IL-13 show no alteration

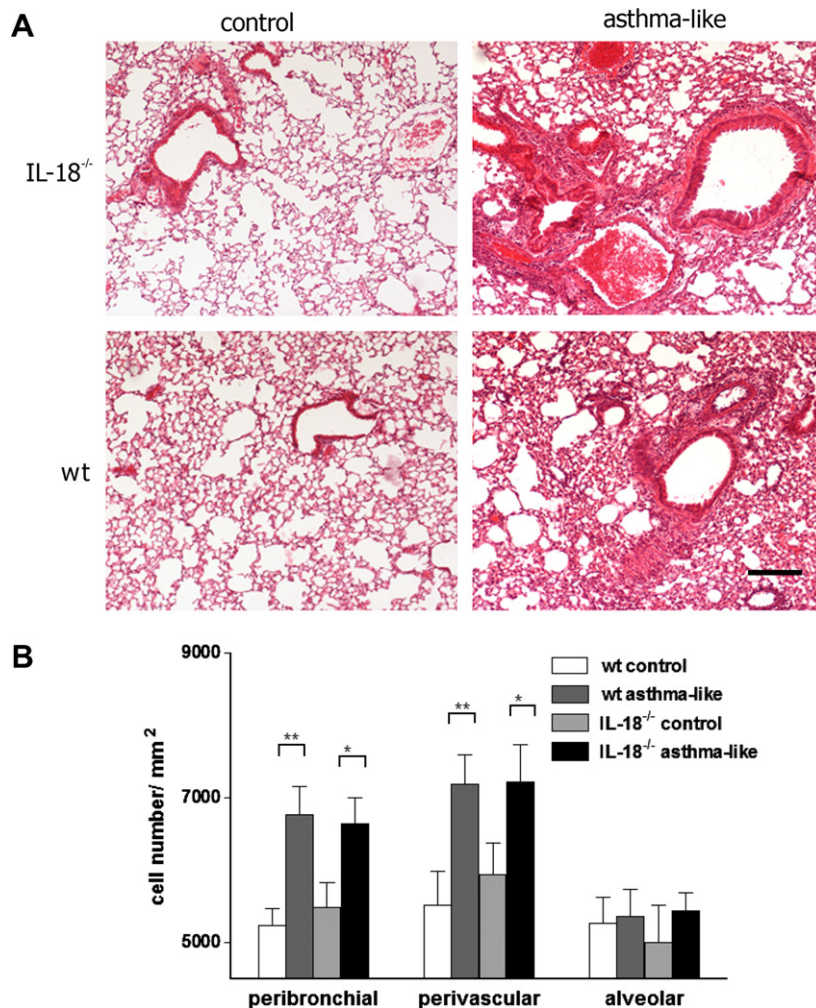


Fig. 3. OVA sensitization and challenge induced perivascular and peribronchial inflammatory infiltrations occur comparably in IL-18^{-/-} and wt mice. (A) Representative microphotographs of H/E-stained lung sections from the four experimental groups as indicated. The bar represents 100 μ m. (B) In sections as demonstrated in (A) cells infiltrating alveolar spaces and perivascular and peribronchial areas were quantified. Shown are mean numbers $\pm$ SEM ($n = 8$ /group) of infiltrating cell obtained from the four experimental groups (* $p < 0.05$, ** $p < 0.01$).

due to induction of asthma (not shown). This might be due to either a restrictive local or a transient expression of these cytokines, which has already been returned to basal levels at 24 h after the last challenge [16].

Surprisingly, we also find the mean IL-17 serum level to be enhanced in the asthmatic IL-18^{-/-} mice (161.7 ± 71.0 pg/ml) in comparison to the IL-18^{-/-} controls (25.0 ± 17.2 pg/ml). This might point to the possible involvement of Th17-cell priming in the course of induction of experimental asthma [17], probably effective only in the absence of IL-18. However, additional experimental work is needed to address this point adequately, which is not subject of this study.

In lung lysates significant differences are found neither for IL-5, IL-10, and IL-13 nor for eotaxin levels. BALf of the four experimental groups contain comparable levels of IL-5, IL-10, and IL-13 (not shown and Fig. 6). In BALf of asthmatic wt mice eotaxin levels seem to be enhanced in compar-

ison to controls, however, without statistical significance. In IL-18^{-/-} mice such a tendency is not observed (Fig. 6).

3.4. Lung physiology is not altered due to absence of IL-18

Aberrant lung function is the main clinical symptom of allergic asthma. In order to analyze the influence of IL-18 on lung physiology airway responsiveness to metacholin (MCh) was determined. Surprisingly, no distinct airway hyperresponsiveness could be found in all experimental groups. Therefore even between the control and the asthma groups no differences were detectable (not shown). However, it is known that AHR is difficult to induce and to measure in C57BL/6 mice [16]. It seems that the body plethysmography used in this study, which works fine in BALB/c mice [18], is not sensitive enough to detect experimental asthma-induced differences of lung physiology in C57BL/6 mice.

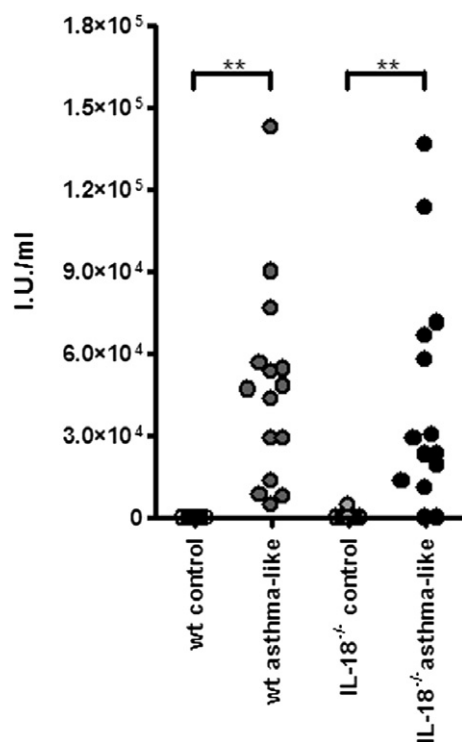


Fig. 4. IL-18 deficiency does not affect enhanced OVA-specific IgE titers due to experimentally induced asthma. Sera obtained from mice as indicated on the x-axis were tested for OVA-specific IgE by ELISA. Shown are individual values for single mice of the four experimental groups ($n = 15/\text{group}$). Values below detection limit of the method (3906.25 IU/ml) are reported as 0 ($^*p < 0.05$, $^{**}p < 0.01$).

4. Discussion

IL-18, a cytokine belonging to the IL-1 family, is considered a pro-inflammatory factor which enhances IL-12-induced IFN- γ production in Th1 cells. It however, is also able to promote a Th2-type immune response when acting in combination with an appropriate cofactor, e.g. IL-4. Its expression in lungs of mice has been shown to be enhanced after induction of asthma with cockroach antigen [10] and in lungs of rats with IgG complex-induced alveolitis [19]. Thus the expression of IL-18 seems to be enhanced locally in the lung upon induction of an inflammatory reaction, in accordance with its proposed pro-inflammatory function. Accordingly we observe an enhanced expression of IL-18 and also IL-18R α in lungs of asthmatic mice in comparison to controls.

Thus ours and other studies point to an involvement of IL-18 in the pathogenesis of experimental asthma. Its role, however, is discussed controversially. Antibody-mediated neutralization of IL-18 prior to allergen challenge accelerated accumulation of eosinophils and AHR in experimental asthma in mice [10], while systemic application of exogenous IL-18 prior to allergenic challenge reduced symptoms of asthma, but only when combined with IL-12 [12]. Locally administered exogenous IL-18-induced asthmatic symptoms in normal mice when combined with IL-2 [8] and in combination with allergen it enhanced

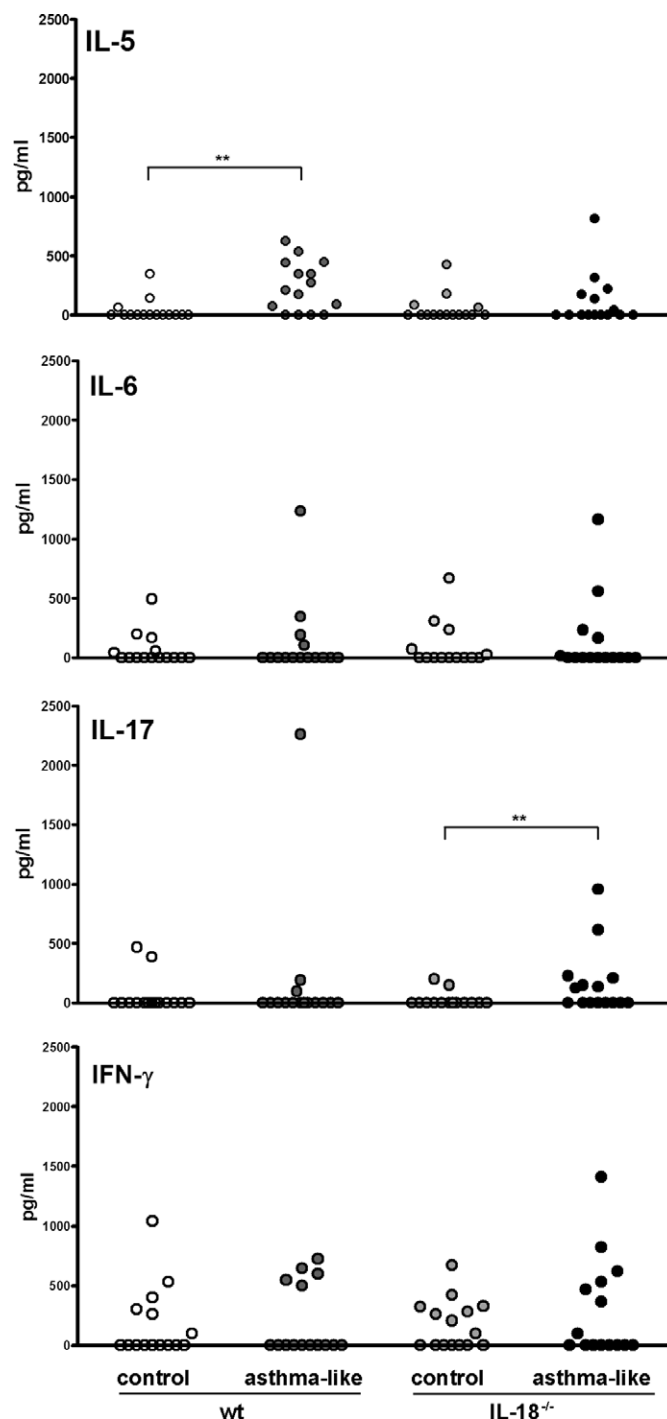


Fig. 5. Serum cytokine levels in control and asthmatic mice of either wt or IL-18 $^{-/-}$ genotype. Sera of mice of the four experimental groups ($n = 15/\text{group}$) as shown on the x-axis were prepared and cytokine concentrations were measured by bead-based multiplex assay. Depicted are the individual values for IL-5, IL-6, IL-17, and IFN- γ of single mice. Values below detection limit of the method (IL-5: 14 pg/ml; IL-6: 27 pg/ml; IL-17: 27 pg/ml; IFN- γ : 14 pg/ml) are reported as 0 ($^*p < 0.05$, $^{**}p < 0.01$).

eosinophilia in sensitized mice [11,20]. Thus, the function of IL-18, especially endogenous IL-18, in asthmatic disease is not well defined, justifying the analysis of the effect of genetic ablation of IL-18 on experimentally induced asthma.

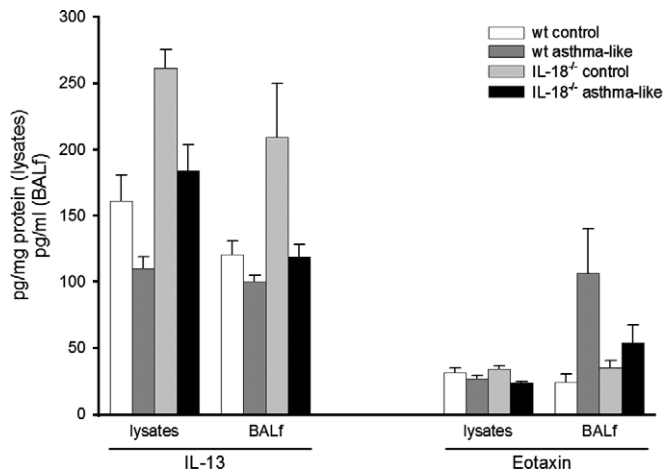


Fig. 6. IL-13 and eotaxin levels in BALf and lung homogenates. BALf and lung homogenates were prepared from mice of the four experimental groups. IL-13 and eotaxin levels were analyzed by ELISA and are presented as indicated on the x-axis. Albeit eotaxin levels in BALf of asthmatic wt mice tend to increase compared to controls, no significant differences were detected (control: $n = 5$ /group; asthma-like: $n = 8$ /group).

In order to address this question we induced asthma by sensitizing and challenging C57BL/6 mice of either the wt or the IL-18^{-/-} genotype with OVA. This resulted in the appearance of asthma-specific parameters such as eosinophilia in BALf, inflammatory infiltration in lung tissue, and allergen-specific serum IgE. In contrast to the method described by Foster et al. [21], who uses a vigorous challenging procedure, the protocol to induce experimental asthma used in this study did not result in AHR. This adds to the notion that C57BL/6 mice are highly resistant to OVA-induced experimental asthma, but focuses the resistance to the lung function, while immunological parameters typical for asthma are readily inducible. Thus, sensitizing and challenging C57BL/6 mice with OVA as described induced a mild asthma-like rather than real asthma disease.

The comparison of wt and IL-18^{-/-} mice demonstrates that none of the parameters indicative for the asthma-like disease was altered due to the absence of IL-18. Only the IL-5 levels in sera and the eotaxin levels in BALf of asthmatic IL-18^{-/-} mice seem to be slightly altered as compared to wt mice. Thus the effect of endogenous IL-18 on the onset of the OVA-induced asthma-like disease in C57BL/6 mice is if at all, negligible.

Our data are in contrast to those published previously [22], demonstrating an increase in IL-5 levels in lung tissue and in lung-infiltrating eosinophils in asthmatic IL-18-deficient mice in comparison to wt mice. Kodama et al. used the Foster method [21] to induce experimental asthma, resulting in about 10 times more eosinophils in BALf than in our study. Thus the very robust infiltration of eosinophils due to vigorous challenging seems to be affected slightly by endogenous IL-18. The inability of detecting an effect of IL-18 on the asthma-like disease in our study might also be explained by the observation, that neutralization of IL-18

at the time of allergenic challenge had a very transient effect on eosinophilia present only within the first hours after challenge [10]. Thus, at 24 h after the last challenge, when we prepared the material for analysis, numbers of eosinophils might already be back at baseline levels.

Our study provides evidence that the increase in serum levels of the Th2-type cytokine IL-5 due to the induction of experimental asthma in C57BL/6 mice, which preferentially mount a Th1-type inflammatory response [23], seems to depend partly on the presence of endogenous IL-18. Upon stimulation with antigen plus IL-18 Th1 cells at least in vitro might develop into ultra-inflaming T cells [7,24] or super Th1 cells [25], able to secrete Th1-type and a limited set of Th2-type cytokines and to induce asthma or atopic dermatitis. Thus, in the C57BL/6 model of OVA-induced experimental asthma, naïve Th cells probably develop mostly into Th2 cells inducing asthmatic symptoms, while the development of ultra-inflaming T cells, which would depend on endogenous IL-18, is of minor relevance for this process.

Acknowledgments

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