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Bronchial Hyperreactivity is Correlated with Increased Baseline Airway Tone

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Abstract
Physiologically, airways are not completely relaxed but maintain a baseline airway tone (BAT). Although not fulfilling the criteria for obstructive airway disease, increased BAT may nevertheless be important because the same amount of airway narrowing can be well tolerated or can cause severe airway obstruction depending on the starting point of the narrowing. In this study, we aimed at studying if BAT is correlated with bronchial hyperreactivity (BHR). For in vitro studies, airways in murine lung slices were digitally recorded and the change in cross-sectional area with time was quantified. BAT was measured by the amount of relaxation induced by permeabilization of the cell membrane with β-escin in zero external calcium. BHR was induced by incubation of lung slices with interleukin-13 (IL-13). T-bet knock-out mice served as an additional model for BHR. T-bet knock-out mice show a shift towards TH2-lymphocytes and display histological as well as functional characteristics of asthma. In vivo, the specific airway resistance of healthy non-smoking volunteers was assessed before and after inhalation of formoterol and bronchial challenge was performed using methacholin. In murine lung slices that had been cultivated without serum, only a minimal BAT could be observed. But, after cultivation with 10 % new born calve serum, airways showed a BAT of ~ 13 % that could be reduced by incubation with an IL-13 receptor antagonist. Atropine, isoproterenol and indomethacin failed to relax airways regardless of cultivation with serum. Incubation of lung slices without serum but with IL-13 increased BAT as well as airway responsiveness to acetylcholine and both effects were more pronounced in small compared to large airways. In lung slices from T-bet knock-out mice, airways were hyperreactive compared to airways in slices from wild type mice and BAT was found to be increased. Again, both effects were more pronounced in small compared to large airways. In human non-smokers without airway obstruction, increased BAT was correlated with bronchial hyperreactivity. We therefore conclude that although not fulfilling the criteria for obstructive airway disease, increased airway tone may yet be relevant in asthma.

Key words: baseline airway tone, bronchial reactivity, lung slices, asthma

List of abbreviations: ASMC = airway smooth muscle cell, BAT = baseline airway tone, BHR = bronchial hyperreactivity, DMEM = Dulbecos' modified eagles medium, DPI = dry powder inhaler, IL-13 = interleukin-13, MCH = methacholin, NANC = nonadrenergic, noncholinergic, NCS = newborn calves' serum, sHBSS = supplemented Hanks' balanced salt-solution, SR_{aw} = specific airway resistance

Introduction

Reports of spontaneous airway smooth muscle cell (ASMC) tone date back to the 1950ies and 1960ies. Klassen and colleagues reported that in humans vagotomy increased bronchial caliber observed through a bronchoscope [17] and subcutaneous injections of atropine as well as inhalation of isoproterenol aerosols decreased airway resistance up to 56 % [6]. In anaesthetized dogs, atropine increased the width of the larger airways up to 20 % [16]. Interpretations what the function of BAT may be reached from being entirely artificial to compulsive waves of changes in BAT augmenting the clearance of the lung comparable to the gastrointestinal tract [36]. More recently, Dates et al. reported an increase in FEV1 of 1.8 % after inhalation of terbutaline in normal adults [7]. In infants, inhalation of albuterol also increased expiratory flow rates [10]. The role of BAT in fetal lung development also has been studied and rhythmic changes in BAT were interpreted to propel fluids through the lungs of the fetal period [33]. Kesler and Canning found BAT in the trachealis of mechanically ventilated guinea-pigs that depended on cholinergic innervation [14] and that was modulated by nonadrenergic, noncholinergic (NANC) activity [15]. Molfino et al. reported that airway tone in humans is mainly vagally controlled using an acoustic reflection technique [29]. On the other hand, Linden et al. report NANC responses to stabilize spontaneous airway tone in the guinea-pig [21-24]. In a comprehensive review, Schmidt and Rabe suggest inherent tone in human isolated bronchi to be the result of a balance between contractile mediators, in particular cysteinyl-leukotrienes, and bronchodilating prostanoids, in particular prostaglandin E_2 [34].

Lung function tests assess the status of airway narrowing and thresholds have been suggested defining manifest airway obstruction. But, changes in BAT without necessarily fulfilling the criteria for obstructive airway disease may also be relevant. Bronchial hy-

perreactivity (BHR) is a key feature of asthma and in a state, where ASMC are more responsive to stimuli, BAT may also be altered. This is even more important given the fact that the same amount of airway narrowing could be well tolerated or could cause severe airway obstruction depending on the starting point of the narrowing. We therefore aimed in the present study on investigating if the degree of BAT and bronchial reactivity are connected.

For in vitro studies, we used murine lung slices. The major advantage of this culture form is that the in situ organization of the lung tissue and the contractility of the ASMC are maintained for several days. Lung slices have been used before to study a variety of lung responses including bronchial contractility [8, 25-28, 32], vascular responses [11] and mucociliary function [19]. Previously, we used lung slices to measure airway responsiveness and intracellular Ca^{2+}-signaling in ASMC [3-5] and we believe that lung slices in combination with video microscopy allow studying BAT in vitro in an in vivo like environment. Addressing the question if data obtained in vitro from mice can be transferred to the in vivo situation in humans, we also included clinical data from lung function tests to demonstrate that murine lung slices in fact serve as a model relevant to clinical findings.

T-bet is a TH1-specific transcription factor and has the ability to direct TH2-cells into TH1-cells [31]. Naïve mice that have been target deleted of the T-bet gene (T-bet knock-out mice) spontaneously develop airway remodeling reminiscent of human asthma and demonstrate multiple functional and inflammatory features characteristic of this airway disease [9]. In our experiments, we used lung slices from T-bet knock-out mice thereby confirming that asthmatic alterations in airway contraction dynamics are preserved in this in vitro model under ex vivo culture conditions.

In this study, we demonstrate that the proinflammatory cytokine IL-13 increases BAT in murine lung slices and that this is accompanied by airway hyperreactivity. In lung slices from T-bet knock-out mice, airways are hyperresponsive to acetylcholine and show an elevated BAT. Both, hyperreactivity and increased BAT are more pronounced in small compared to large airways. In non-smoking humans without airway obstruction, the level of bronchial reactivity correlates with the extent of BAT. We therefore conclude that BHR is associated with increased BAT and that increased airway tone without fulfilling the criteria for obstructive airway disease may yet be relevant in asthma.

Material and Methods

In vitro Experiments

Cell culture reagents were obtained from Life Technologies (Eggenstein, Germany), other reagents from Sigma (Deisenhofen, Germany). Balb/C mice were purchased from Harlan-Winkelmann (Borchen, Germany) and T-bet knock-out mice on a Balb/C background from Charles River (Charles River Breeding Labs, Needham, MA). All procedures had been approved by the Ethics Committee of the Ludwig-Maximilians-University, Munich.

Lung slices were prepared as described previously [3]. Briefly, mice 42 to 77 days old were sacrificed by intraperitoneal injection of Nembutal and the chest wall was removed. The trachea was cannulated using an intravenous catheter and the lungs were inflated with 2% agarose-sHBSS at 37 °C. Subsequently, 0.1 to 0.2 ml of air was injected to flush the agarose-sHBSS out of the airways and the agarose was gelled by placing the mouse preparation at 4 °C. The lungs were removed and slices of ~200 µm thickness were cut with an EMS-4000 Tissue Slicer (Electron Microscopy Sciences, Fort Washington, PA). The slices were maintained by floating them in DMEM supplemented with antibiotics and antimycotics at 37 °C in 5 % CO_2 for up to 5 days. In culture, lung slices maintain their contractile properties for up to one week [3]. Experiments were therefore performed on day 2 to 5 of cultivation and each slice was used for one experiment only. For each experimental group, slices from at least 3 different mice were used. To measure airway cross-sectional area, lung slices were placed in culture dishes immersed in sHBSS and held in position by a piece of a custom made gold grid. Bright field images were recorded using a digital CCD camera (AxioCam MRm, Carl Zeiss Vision, Munich, Germany). Frames were captured in time-lapse (0.5 frame/s) and the cross-sectional area of the airway was measured by pixel summing using the image analysis software "Scion" (Scion Corporation, Frederick, Maryland).

To measure BAT, lung slices were briefly washed in phosphate-bufferd saline without calcium containing 0.02 % EDTA and placed on the microscope stage immersed in the same solution. After starting the recording, a solution containing β-escin dissolved in DMSO and ATP was added to the dish resulting in final concentrations of 100 mM β-escin and 100 nM ATP in phosphate-bufferd saline without calcium containing 0.02 % EDTA. β-escin permeabilizes the cell membrane and in a Ca^{2+}-free solution, the lack of calcium leads to ASMC relaxation.

To compare airways of different sizes, small airways were defined as airways with a cross-sectional area less than the median of all airways measured (20427 μm^2), whereas large airways enclosed a larger cross-sectional area.

To quantify spontaneous oscillations in BAT, airways in lung slices were recorded for 10 min immersed in sHBSS. According to preliminary experiments, changes of > 1 % in cross-sectional area were regarded significant.

Assessment of SR_{aw} in Humans

All procedures had been approved by the Ethics Committee of the Ludwig-Maximilians-University, Munich and all volunteers had given informed consent. Lung function tests were performed using a Compact Lab body plethysmograph (Jäger, Würzburg, Germany). Formoterol was inhaled as DPI using an Oxis Turbohaler® (Astra Zeneca, London, UK) and placebo inhalation was performed using a Turbohaler® without content. Bronchial challenge was performed using a 3-step inhalation protocol delivering cumulatively 0.65 mg methacholin (MCH, Apotheke Klinikum-Innenstadt, Munich, Germany).

Statistics

The "ANOVA" or "ANOVA repeated measurements" tests (combined with an all pair-wise multiple comparison procedure) were used. To test for correlations, a polynomial regression analysis using the Sigma Stat software (Jandel Scientific, Chicago, IL) was performed. A P value of $P < 0.05$ was considered statistically significant.

Results

Baseline Airway Tone in Mouse Lung Slices

Lung slices were cut 200 μm thick and cultivated for up to 5 days. Without prior addition of contractile agonists, exposure to the β-escin-relaxation solution caused a minor increase in cross-sectional area of 2.05 ± 0.6 % of starting value (mean ± SEM, n = 21, Fig. 1). Cultivating the slices in DMEM supplemented with 10 % new born calves' serum (NCS), exposure to the β-escin-relaxation solution resulted in a increase in cross-sectional area of 13.2 ± 1.6 % of starting value (n = 21, $P < 0.05$ versus cultivation without serum, Fig. 1). In some cases, a contraction (decrease in cross-sectional area) was observed following the relaxation. Serum contains proinflammatory cytokines including IL-13 and this interleukin is elevated in the serum of patients with asthma [2, 20]. To test if the increased BAT was caused by IL-13 in the serum, slices were cultivated for 24 h with serum in the presence of an IL-13-receptor blocker (IL-4Rα-antibody, R&D Systems, Minneapolis, MN). In these experiments, BAT was found to be decreased compared to the cultivation with serum alone (5.8 ± 1.3 % with the IL-4Rα-antibody versus 13.2 ± 1.6 % without the antibody, n = 21, $P < 0.05$, Fig. 1). To determine whether BAT was based on cholinergic, adrenergic or cyclooxygenase activity, the influence of atropine, isoproterenol and indomethacin on BAT in slices that had been cultivated with serum was measured. The relaxing potential of all 3 substances was considerably less compared to the β-escin-relaxation solution (13.2 ± 1.6 % of starting value for β-escin, 1.9 ± 0.5 for atropine, 2.2 ± 0.7 for isoproterenol, 1.1 ± 3.2 for indomethacin, n = 8, $P < 0.05$ for β-escin versus all other groups, Fig. 2). Recording airways in lung slices without the addition of agonists, 21.4 % of all airways showed spontaneous oscillations in BAT. These oscillations displayed an average frequency of 0.6 ± 0.2 min^{-1} and an amplitude of 6.2 ± 0.9 % of starting value (n = 21, Fig. 3).

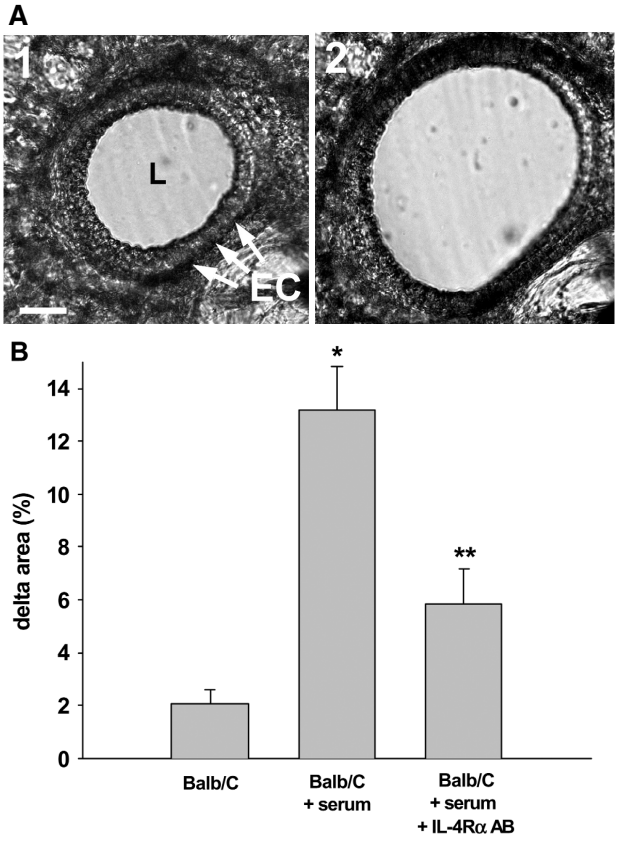

Fig.1. (A) Bright field images of β-escin induced bronchial relaxation. Murine lung slices were cut 200 μm thick, cultivated for up to 5 days and the cross-sectional area of the airways in the slices was digitally recorded. When cultured with 10 % NCS, exposure to a β-escin-relaxation solution caused bronchial dilatation. (1: immediately before, 2: 15 sec after addition of β-escin, L = airway lumen, EC = epithelial cells, bar = 25 μm). (B) β-escin induced bronchial relaxation. The β-escin-relaxation solution caused a minor increase in cross-sectional area of about ~ 2 % of starting value. Cultivating the slices in DMEM supplemented with 10 % NCS (Balb/C + serum), exposure to a β-escin-relaxation solution resulted in a increase in cross-sectional area of ~ 13 % of starting value. To test if the increased BAT was caused by IL-13 in the serum, slices were cultivated for 24 h with serum in the presence of an IL-13-receptor blocker (Balb/C + serum + IL-4Rα AB). In these experiments, BAT was found to be decreased to ~ 6 % (n = 21, * = $P < 0.05$ versus all other groups, ** = $P < 0.05$ versus Balb/C + serum).

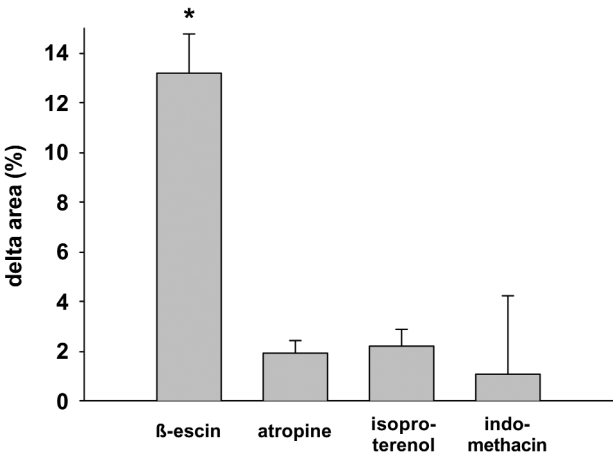

Fig. 2. Regulation of baseline airway tone. Analyzing the cross-sectional area of airways in lung slices that had been cultivated with serum, the influence of atropine, isoproterenol and indomethacin on BAT was assessed. The relaxing potential of all 3 substances was considerably less compared to the β-escin-relaxation solution (n = 8, * = $P < 0.05$ versus all other groups).

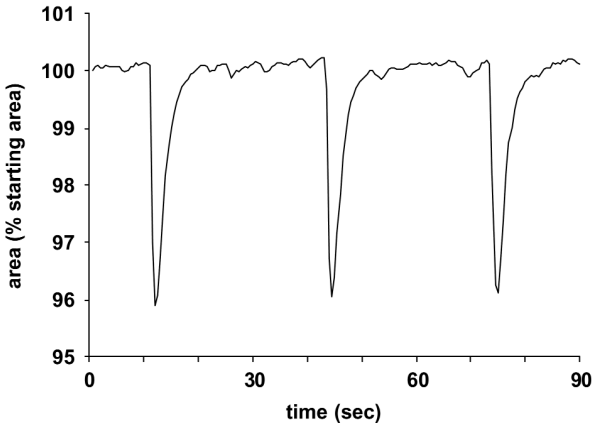

Fig. 3. Representative trace of spontaneous oscillations in baseline airway tone. Recording lung slices without the addition of agonists, 21.4 % of the airways showed spontaneous oscillations in BAT consisting of a contraction (decrease in cross-sectional area) followed by a relaxation (increase in cross-sectional area).

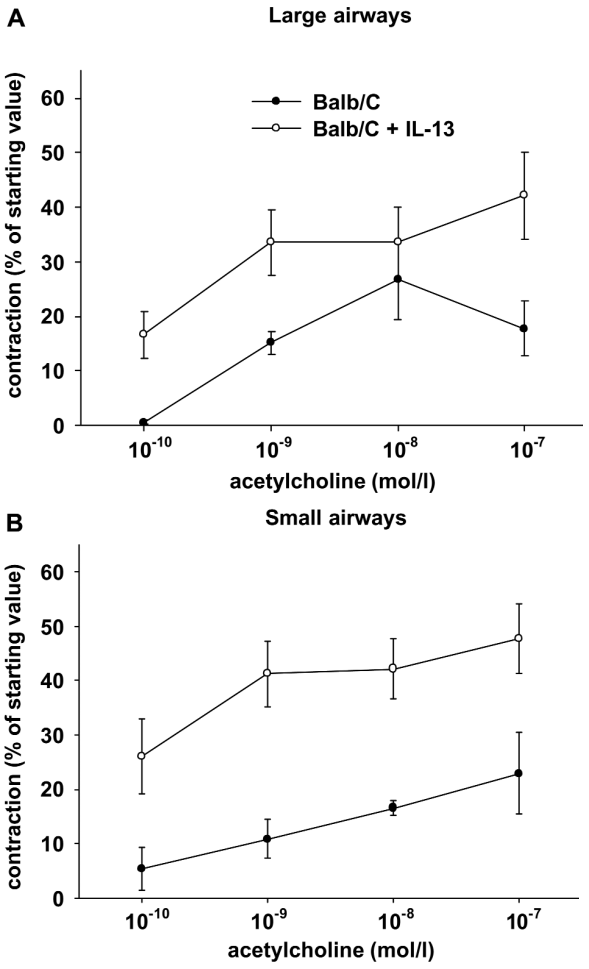

Fig. 4. Impact of IL-13 on bronchial reactivity in mouse lung slices. Airways in lung slices were cultivated with 20 ng/ml IL-13 for 24 h, exposed to increasing concentrations of acetylcholine and the decrease in cross-sectional area was measured. Large (A) and small (B) airways that had been cultivated with IL-13 were found to contract to a greater extend compared to airways without IL-13. This effect was more pronounced in small airways (n = 6 – 12 for each data point, $P < 0.01$).

Impact of IL-13 on Bronchial Reactivity and BAT in Mouse Lung Slices

Airways in lung slices were exposed to increasing concentrations of acetylcholine and the decrease in cross-sectional area was measured. After incubating without serum but with 20 ng/ml IL-13 for 24 h, bronchial responsiveness was increased, both in small and large airways with the effect being higher in small airways ($P < 0.01$, Fig. 4). In addition, BAT was elevated by the incubation with IL-13 (6.8 ± 1.9 % with IL-13 compared to 2.05 ± 0.6 % without IL-13, n= 8, $P < 0.05$, Fig. 5A). Small airways showed a tendency towards higher increase in BAT compared to large airways (8.9 ± 3.3 % in small versus 5.3 ± 2.1 % in large airways, Fig. 5B).

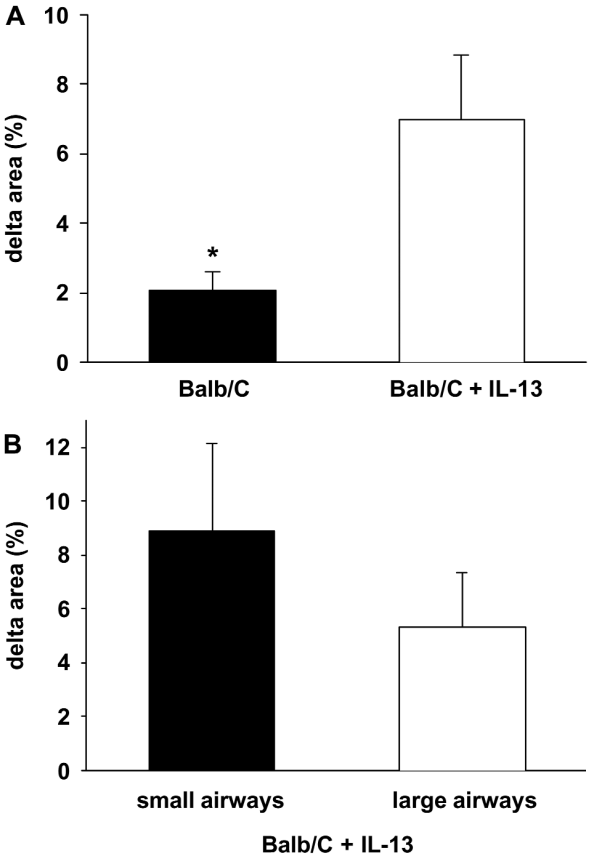

Fig.5. Impact of IL-13 on BAT in mouse lung slices. (A) Cultivation of lung slices from Balb/C mice for 24 h with 20 ng/ml IL-13 (Balb/C + IL-13, white column) augmented BAT as assessed by β-escin induced relaxation compared to the cultivation without IL-13 (Balb/C, black column, n = 17, * = $P < 0.05$). (B) Comparing small (black column) and large airways (white column), no significant differences could be found although small airways showed a tendency towards higher BAT.

Bronchial Responsiveness and BAT in Lung Slices from T-bet knock-out mice

T-bet knock-out mice display characteristic features of asthma including TH2-based airway inflammation [9].

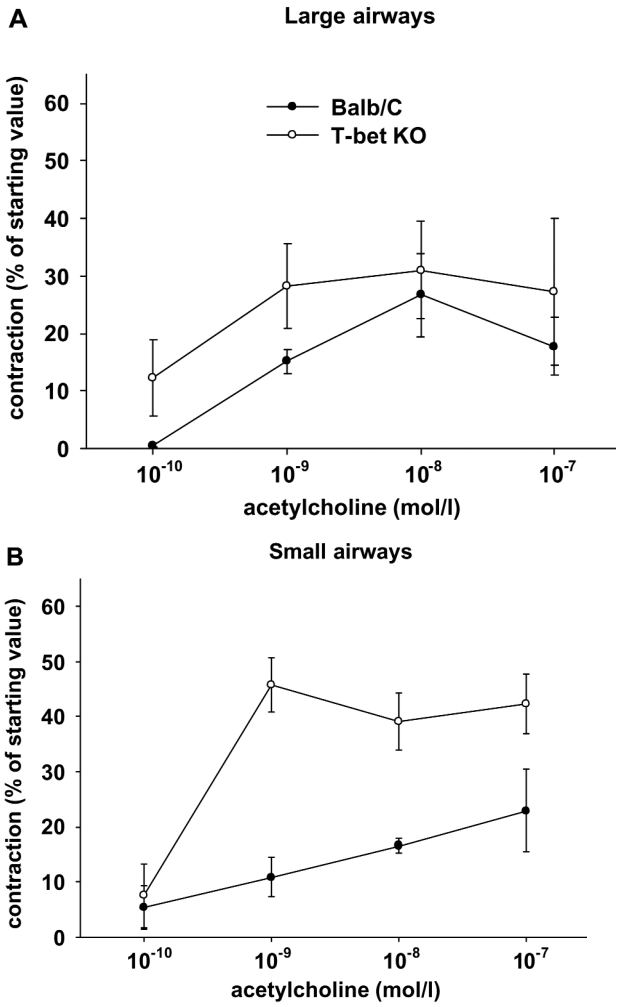

Fig.6. Bronchial reactivity in lung slices from T-bet knock-out mice. Airways in lung slices were exposed to increasing concentrations of acetylcholine and the decrease in cross-sectional area was measured. Large (A, $P < 0.05$) and small (B, $P < 0.01$) airways from T-bet knock-out mice were found to contract to greater extend compared to airways from Balb/C wild type mice. This effect was more pronounced in small airways ($n = 6 – 10$ for each data point, $P < 0.05$).

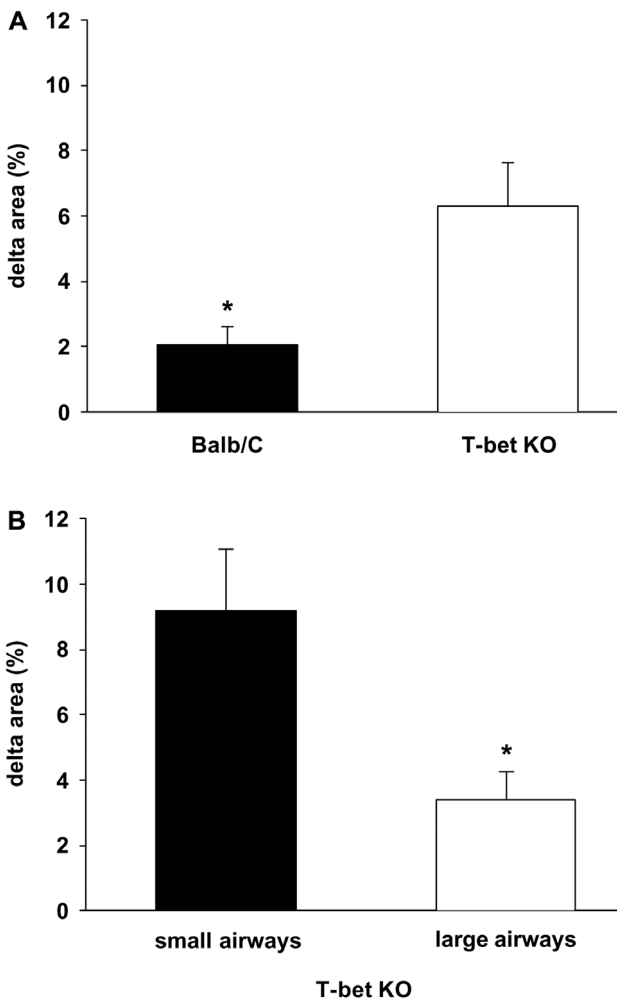

Fig. 7. BAT in lung slices from T-bet knock-out mice. (A) BAT was assessed by β-escin induced relaxation of airways in lung slices. Airways in slices from T-bet knock-out mice (T-bet KO, white columns) showed an increased BAT compared to airways in slices from wild type mice (Balb/C, black columns). (B) Comparing small and large airways in lung slices from T-bet knock-out mice, BAT was found to be higher in small airways ($n = 15$ - 21, * = $P < 0.05$).

In lung slices from T-bet knock-out mice, acetylcholine-induced airway contraction was increased in large (Fig. 6A, $P < 0.05$) and small (Fig. 6B, $P < 0.01$) airways compared to slices from wild type mice. These differences were more pronounced in small airways. BAT in airways from T-bet knock-out mice was also found to be elevated compared to wild type mice (2.1 ± 0.6 % in wild type versus 6.3 ± 1.3 in T-bet knock-out mice, $n = 15$, $P < 0.05$, Fig. 7A). Comparing small and large airways, these effects were more pronounced in small airways (9.2 ± 1.9 % in small versus 3.4 ± 0.8 % in large airways, $P < 0.05$, Fig. 7B).

Spontaneous Oscillations in BAT in T-bet knock-out Mice and after Incubation with IL-13

Quantifying spontaneous oscillations in BAT, no influence of cultivation with or without serum could be found. Incubation of lung slices with 20 ng/ml IL-13 for 24 h augmented the percentage of airways displaying oscillations (Table 1). Airways in lung slices from T-bet knock-out mice showed a higher frequency of the oscillations compared to the wild type.

Baseline Airway Tone in Humans

Healthy non-smokers without a history of asthma ($n = 8$, 5 female and 3 male, age 37 ± 3 years, mean ± SEM) underwent lung function testing. Despite normal starting values for specific airway resistance (SR_{aw}), all volunteers inhaled placebo and formoterol. Inhalation of placebo had no effect (Fig. 8A) while inhalation of 36 µg formoterol decreased SR_{aw} (0.74 ± 0.1 kPa*sec before versus 0.58 ± 0.1 kPa*sec after 36 µg formoterol, $P < 0.05$, Fig. 8B). A second inhalation cumulatively delivering 96 µg formoterol had no further effect.

Table 1. Spontaneous oscillations in baseline airway tone in lung slices.

	Balb/C	Balb/C + IL-13	T-bet
airways showing oscillations (%)	21.4	81.2 *	16.7
frequency of oscillations (min^{-1})	0.6 ± 0.2	1.7 ± 0.2	5.3 ± 1 **
amplitude of oscillations (% of starting value)	6.2 ± 0.9	8.6 ± 2.9	3.0 ± 0.4

n = 15 – 21, all groups cultivated without serum
* = P < 0.05 versus all other groups
** = P < 0.05 versus Balb/C

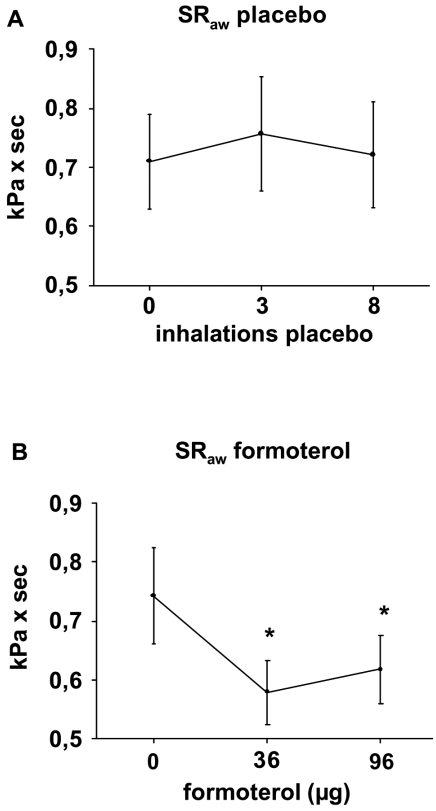

Fig. 8. Bronchodilation in healthy non-smokers. In healthy non-smokers without a history of asthma specific airway resistance (SR_{aw}) was assessed before and after inhalation of placebo and formoterol. (A) Inhalation of placebo had no effect on SR_{aw}. (B) Inhalation of 36 µg formoterol decreased SR_{aw} whereas a second inhalation delivering cumulatively 96 µg formoterol showed no further effect. n = 8, * = P < 0.05 versus starting value.

Correlation of Baseline Airway Tone and Bronchial Reactivity in Humans

Healthy non-smoking volunteers (n = 15, 8 female and 7 male, age 32.2 ± 2.5 years, mean ± SEM) underwent lung function testing, inhalation of 36 µg formoterol as well as bronchial challenge with 0.65 mg MCH. The increase in SR_{aw} caused by MCH correlated with the decrease in SR_{aw} caused by formoterol indicating a correlation of bronchial responsiveness with BAT (R = 0.56, P < 0.05, Fig. 9).

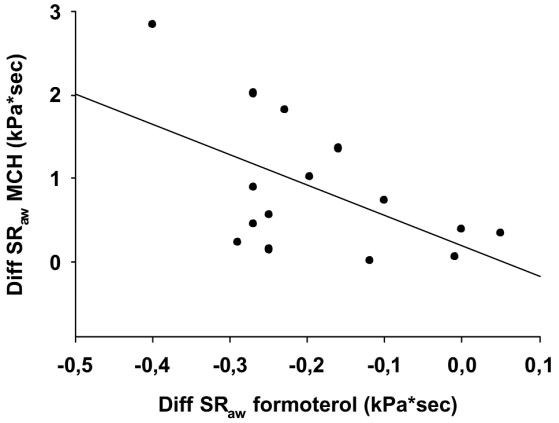

Fig. 9. Correlation of bronchial reactivity and BAT in humans. Non-smoking volunteers underwent lung function testing, inhalation of 36 µg formoterol and bronchial challenge with 0.65 mg MCH. The increase in SR_{aw} caused by MCH correlated with the decrease in SR_{aw} caused by formoterol indicating a correlation between bronchial reactivity and BAT (n = 15, R = 0.56, P < 0.05).

Discussion

In murine lung slices, the proinflammatory cytokine IL-13 increased BAT and this was accompanied by airway hyperreactivity. In lung slices from T-bet knock-out mice, airways showed an elevated BAT and were hyperreactive to acetylcholine. Both hyperreactivity and increased BAT were more pronounced in small compared to large airways. In healthy humans without airway obstruction, the extent of BAT correlated with bronchial reactivity. We therefore conclude that BHR is associated with increased BAT.

T-bet is a TH1-specific transcription factor that has the ability to redirect TH2-cells into TH1-cells [31]. Mice with a target deletion of the T-bet gene show a functional and histological phenotype similar to that created by allergen exposure in sensitized mice [9]. In particular, the airway remodeling these mice develop spontaneously is reminiscent of human asthma. Lung slices from T-bet knock-out mice maintain their morphology in culture for several days (data not shown) and we found airways in lung slices from these mice to be hyperresponsive to acetylcholine. Therefore, T-bet knock-out mice lung slices constitute a useful in vitro model to study asthmatic alterations in airway contraction dynamics.

To explore BAT of airways in lung slices independently from pharmacological properties, we used β-escin to permeabilize the cell membrane of the ASMC. Calcium is an important intracellular second messenger leading to ASMC contraction. After permeabilization of the cell membrane in a Ca^{2+}-free solution the lack of calcium led to ASMC relaxation. The airway contraction following the relaxation observed in some experiments probably reflects the permeabilization of the sarcoplasmic reticulum releasing the calcium stored therein. After washing with sHBSS, acetylcholine still induced airway contraction indicating that the treatment with the β-escin relaxation solution did not cause significant damage to the ASMC (data not shown).

The mechanisms by which BAT is regulated have been studied to some extent. Kesler and Canning found BAT in the trachealis of guinea-pigs to be dependent on cholinergic innervation [14] and to be modulated by NANC activity [15]. Molfino et al. reported that airway tone in humans is vagally controlled [29]. On the other hand, Linden et al. showed NANC responses to stabilize spontaneous airway tone in the guinea-pig [21-24]. Dales and colleagues reported a 1.8 % increase in FEV1 of healthy subjects after inhalation of terbutaline [7]. In our study, we found in vivo a decrease in SR_{aw} of ~ 20 % after inhalation of formoterol. In vitro, neither atropine, isoproterenol nor indomethacin caused significant airway relaxation suggesting that in murine lung slices BAT is not caused by cholinergic or adrenergic pathways nor by cyclooxygenase products. Our data indicate that BAT is at least partially caused by inherent properties of the ASMC themselves. For instance, spontaneous intracellular Ca^{2+}-events like Ca^{2+}-puffs or Ca^{2+}-sparks intermittently lead to whole cell events like intracellular Ca^{2+}-waves and these waves cause contraction. Inflammatory influences may increase the frequency of spontaneous Ca^{2+}-events and thereby increase BAT. However, this hypothesis was beyond the scope of the present study but will be addressed in future studies using lung slices with 2-Photon-microscopy.

Because cultivation of lung slices with serum increased the degree of BAT, factors within the serum obviously influenced the baseline tone. The addition of an IL-4Rα-antibody blocking the IL-13 receptor diminished the serum-dependent increase in BAT. However, the degree of BAT was still higher compared to the cultivation of lung slices without serum. Therefore, additional factors within the serum like leukotriens or interleukins besides IL-13 probably added to BAT.

Rhythmic changes in airway tone have been reported before including observations in humans during bronchoscopy [13] and on X-radiographs [30] as well as in isolated guinea-pig trachea [35]. In our study, ~ 20 % of the airways displayed spontaneous oscillations in BAT leading to measurable changes in cross-sectional area. Furthermore, spontaneous activity of parts of the bronchial wall without significantly changing the cross-sectional area frequently could be observed. These "twitches" presumably represented single cell contractions while coordinated contraction of several ASMC led to airway narrowing. The reason why sometimes a contracting cell communicated with surrounding ASMC and sometimes did not remains to be elucidated. But, after incubation with IL-13, the percentage of airways showing coordinated contraction increased. Speculatively, increased gap-junctional coupling between ASMC may explain the increase in cell-cell communication caused by IL-13. Interestingly, although the cultivation with serum influenced the degree of BAT in lung slices, no effect of incubation with serum on the spontaneous oscillatory changes in BAT could be found. Therefore, the influences making airway ASMC exhibiting a BAT and the influences making them changing the degree of BAT in an oscillatory pattern may be different. Airways in lung slices from T-bet knock-out mice showed an increased frequency of the oscillations in BAT but not an increased percentage of airways displaying oscillations. This discrepancy to the effects of IL-13 indicates that the asthma-like alterations seen in T-bet knock-out mice are to complex to be explained by the effect of a single interleukin like IL-13.

For some time, asthma was believed to be merely a large-airway disease but later on, small airways were found to also considerably contribute to asthma [12, 18]. In lung slices from T-bet knock-out mice, bronchial hyperreactivity and increased BAT were more pronounced in small compared to large airways and this further emphasizes the importance of small airways in asthma.

Airway obstruction as being evident by increased airway resistance clearly indicates a severe state of asthma. But, increased BAT without fulfilling the criteria of obstructive airway disease may also play a role because the same amount of airway narrowing can be well tolerated or can cause severe airway obstruction depending on the starting point of the narrowing and this starting point is the BAT. Therefore, the coincidence of elevated narrowing, e.g. bronchial hyperreactivity, with a higher starting point of the narrowing, e.g. increased BAT, would lead to amplified airway obstruction. In our study, IL-13 caused both, bronchial hyperreactivity and increased BAT. Airways in lung slices from T-bet knock-out mice also were hyperresponsive to acetylcholine and showed elevated BAT. The fact that both effects were more pronounced in small compared to large airways further underlines the close connection between the sensitivity to stimuli and the amount of baseline tone. Furthermore, BAT as assessed in vivo by the decrease in SR_{aw} after inhalation of formoterol correlated with bronchial reactivity. These data suggest that the degree of airway narrowing caused by contractile stimuli and the starting point of the narrowing are closely related. Therefore, the detection of airway narrowing in an early state not exceeding the thresholds that define manifest airway obstruction could provide additional information. Furthermore, the role of baseline airway tone in asthma patients without manifest airway obstruction may have been underestimated.

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