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Effects of Ionized Waterfall Aerosol on Pediatric Allergic Asthma

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Objective. Ionized water aerosols have been suggested to exert beneficial health effects on pediatric allergic asthma. Their effect was evaluated in a randomized controlled clinical trial as part of a summer asthma camp. *Methods*. Asthmatic allergic children ($n = 54$) spent 3 weeks in an alpine asthma camp; half of the group was exposed to water aerosol of an alpine waterfall for 1 hour per day, whereas the other half spent the same time at a "control site". Immunological analysis, lung function testing, and fractional exhaled nitric oxide (FeNO) testing were performed during the stay, and sustaining effects were evaluated 2 months later. Symptom score testing was done over a period of 140 days. *Results*. The water aerosol group showed a significant improvement in all lung function parameters, whereas only the peak expiratory flow improved in the control group. All patients showed a significant improvement in symptom score and a significant decrease in FeNO after the camp. Only the water aerosol group exhibited a long-lasting effect on asthma symptoms, lung function, and inflammation in the follow-up examination. Induction of interleukin (IL)-10 and regulatory T (Treg) cells was measured in both groups, with a pronounced increase in the water aerosol group. IL-13 was significantly decreased in both groups, whereas IL-5 and eosinophil cationic protein were decreased only in the water aerosol group. *Conclusions*. Our findings confirm the induction of Treg cells and reduction in inflammation by climate therapy. They indicate a synergistic effect of water aerosols resulting in a long-lasting beneficial effect on asthma symptoms, lung function, and airway inflammation.

Keywords allergic asthma, FeNO, high altitude, ionized water aerosol, lung inflammation, Treg cells, waterfall

INTRODUCTION

Allergic asthma is the most common type of asthma, affecting both children and adults, and it correlates with allergies in about 90% of children and about 50% of adult onset asthmatics (1). It is a complex disease with a strong genetic component (2), and it is characterized by recurrent episodes of wheezing, variable airway obstruction, and bronchial hyperreactivity (BHR) (3, 4). The prevalence of asthma in Austria amounts to 6% of adults and 11% of children (5, 6).

Allergic asthma depends on the presence of Immunoglobulin E (IgE) antibodies in the lung, which trigger stimulation of eosinophilic inflammation of the airways after allergen exposure. Infiltrating T helper cell 2 (TH2) cells orchestrate the inflammation by producing specific pro-inflammatory cytokines such as interleukin (IL)-4, IL-5, IL-13, and tumor necrosis factor, thus inducing recruitment and survival of eosinophils and mast cells and leading to BHR (7, 8). In human asthma, bronchoconstriction is assumed to be triggered by TH2-associated mediators from mast cells, such as IL-9 and IL-13, which can change the excitability of bronchial smooth muscle cells to various stimuli (9).

In the past decades, allergic immune reactions have been explained by an imbalance between TH2 and TH1 cells, but recently, T regulatory (Treg) cells have also been shown to play a role in the context of allergy. These cells, acting via the cytokines IL-10 and/or transforming growth factor-β, can control TH2-type immune responses; and similar to the TH2/TH1 hypothesis, an imbalance between TH2 and Treg cells can result in atopy.

The current status of asthma treatment is focused on standard symptom medication with nonspecific pharmaceuticals, such as leukotriene antagonists, beta-2 agonists, and inhaled corticosteroids (10, 11).

Next to pharmacological therapies, travels to and/or stays at high altitude have a long tradition in the therapy of asthma and have been described to reduce asthma symptoms and to induce immunological changes with positive long-term effects (12–14). Children living in an altitude between 800 and 1200 m have a lower prevalence of bronchial asthma, fewer days of absence from school, and fewer nights with dyspnea due to asthma (15), implying an inverse relationship between residential altitude and asthma (16). Interestingly, high-altitude climate therapy has been shown to have beneficial effects on patients with severe allergic and also intrinsic asthma as reviewed by Rijssenbeek-Nouwens and Bel (17).

Martin Gaisberger and Renata Šanović as well as Markus Ritter and Arnulf Hartl contributed equally to this work.

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Major factors responsible for this positive effect of moderate- and high-altitude climate therapies are a shortened pollen season, allergen avoidance, decreased air pollution, increased ultraviolet radiation, and elevated blood cortisol and catecholamin levels (18–23).

Moreover, high-altitude climate therapy has been found to trigger immunomodulatory mechanisms, as indicated by a study showing alterations of the TH2/Treg cell ratio and an increase in IL-10-producing peripheral blood mononuclear cells (PBMCs) (13).

Besides the parameter "high altitude," waterfalls are specific for the European mountain regions. The typical "waterfall environment" can be characterized by negatively charged and respirable nano- and micro-water aerosols in the range of 0.4–600 nm (24, 25).

The Krimml Waterfalls, the selected site for our study, is one of the biggest European waterfalls with a drop height of 380 m and is located between 1100 and 1480 m altitude and comprises three cascades. The environment of the waterfall has previously been thoroughly characterized using various physical assays and methods (25).

The present randomized controlled study investigates the established high-altitude climate therapy combined with the influence of the waterfall environment (inhaling of ions and aerosols generated by splashing of water termed "ionosols") on pediatric asthma. Moreover, we studied the long-lasting effect of the combined therapy on functional and immunological parameters of allergic asthma in a subgroup of patients in a 4 months follow-up study.

Considering the high and still rising annual costs of asthma medication, an exploration of natural health resources like altitude and ionosols for asthma treatment may help to discover effective, sustainable, and affordable therapies mitigating symptoms and decreasing medication, thus improving the quality of life of patients.

METHODS

Subjects

Fifty-four children from Austria and Bosnia/Herzegovina (8–15 years old) were enrolled in the study. The children were recruited by local pediatricians or lung specialists and suffered from partially controlled to controlled asthma, according to global initiative for asthma (GINA) guidelines (11). All children were given the minimum medication to achieve adequate asthma control with inhaled corticosteroids and beta-sympathomimetics on demand; none of them were treated with systemic corticosteroids. The study was approved by the local ethical committee and informed consent was given by a guardian. The trial was registered at International Standard Randomized Controlled Trial Numbers (ISRCTN) (http://www.controlled-trials.com/ISRCTN04002573).

Study Design

The study was set up as a randomized controlled pediatric clinical study. Patients using long-acting beta-sympathomimetic drugs were excluded.

All children spent a 3-week sojourn at the village of Krimml (Province Salzburg, Austria) located 1067 m above sea level. They were cared for by six primary school teachers. The classrooms of the local primary school were adapted as accommodation; the wooden floored rooms were subdivided into four bedroom compartments. All children received the same standard meals (without peanut products) provided by three local hotels in weeklong shuffles. All children had the same daily routine, including a children-adapted touristic program, focusing on the surrounding national park. Tours were undertaken at an altitude of 900–2250 m with an average of about 1500 m above sea level. Randomization was computed in blocks of four with an equal treatment allocation ratio. For 1 hour each day, the groups were separated for intervention into a control group and a water aerosol group; both groups comprised an equal number of Austrian and Bosnian children. The group separation was kept identical throughout the study.

Intervention

The Krimml Waterfalls with an absolute fall height of 380 m consists of three cascades (140, 100, 140 m). The water aerosol group probands ($n = 27$) spent the hour of intervention at the lowest cascade of the waterfall on the orographic right side of the river about 55–65 m away from the water impact zone (N47° 12′29.80″ E12° 10′14.99″). The control group probands stayed at a control place located about 2.3 km linear distance from the water aerosol group at the same altitude (Figure 1). Moreover, march distance and march height profile with respect to the accommodation of both groups were identical to exclude bias due to different training effects. The 3-week long "Splash Camp" took place from July to August 2007; the follow-up examinations were performed until December 2007.

Environmental Parameters—Pollen Monitoring, Air Ion, and Aerosol Measurements

A Burkhard pollen trap (Burkhard Manufacturing Co. Ltd., Hertfordshire, UK) was installed on top of the roof of the primary school (accommodation) in order to monitor the environmental pollen load during the study. The analysis for tree, grass, and herb pollen and mold was performed by experts of the pollen warning service at the department of the province government of Carinthia.

Air ions are charged airborne particles that are standard constituents of the lower troposphere. They are divided by size diameter into two basic classes: cluster ions (0.36–1.6 nm) and aerosol ions (1.6–79 nm), where the most prominent is the subclass of "small cluster ions" ranging from 0.39 to 0.85 nm (26). Globally, ions are generated by cosmic radiation and nuclear decay of radioactive elements in the air (radon—^{222}Rn) and ground, whereas neutralization is ascribed mostly to attachment to aerosol particles (97%). Background concentration of small ions is about a few hundred per cubic centimeter of each polarity and they are mostly single charged ($\pm 1.6 \times 10^{-19}$ C).

Locally, ions are generated by waterfalls through the process of falling water splashing onto solid (rocks) and aqueous surfaces, inducing charge separation. Ions created

FIGURE 1.—Left photograph: site of the water aerosol group; right photograph: site of the control group.

this way are called "Lenard ions" (27). They are predominantly negatively single charged with a maximal diameter of about 2 nm (24, 25) and concentrations of up to 10^5 ions/cm^3 near waterfalls.

Three identical air-ion detectors (CDI-06) based on the Gerdien aspirated condenser principle (28) were utilized for an ion measuring campaign at Krimml Waterfalls. CDI-06 is a fully automated and programmable measuring instrument equipped with sensors for temperature, humidity, and pressure. Confident and accurate operation is provided using "zeroing" function (29), whereas current leakage due to high humidity is suppressed by a special electrode hanging system. Instruments were set to measure different ion diameters (1, 1.5, and 2 nm) in order to distinguish waterfall generated ions from those generated by background ionization. Wind speed was determined by a PCE-007 anemometer (PCE, Southampton, UK).

Micro-aerosols and nano-aerosols were measured by a Grimm Optical Particle Counter 1.108 and Grimm SMPS+C (Scanning Mobility Particle Sizer + Condensation Nucleus Counter), respectively (Grimm, Mitterfelden, Germany). Measurements were performed at both, the site of the water aerosol exposure and the site where the control group was located.

Ion concentration gradients at Krimml Waterfalls and ion size distribution from 0.9 to 350 nm are described in detail in Kolarz et al. (25).

Experimental Schedule

Table 1 shows the diagnostic investigations and the experimental schedule of the randomized controlled clinical trial. Due to the long travel distance to the laboratory, the 2-month follow-up examination of fractional exhaled nitric oxide (FeNO) and lung function was performed with only 22 children (12 control; 10 waterfall), and the 4-month follow-up period of Childhood Asthma Control Test (C-ACT) included 40 out of 54 patients (all German-speaking children, 20 control; 20 waterfall).

Asthma Control Test Questionnaire

The German version of the C-ACT Questionnaire (http://www.asthmakontrolltest.de) was administered only to German-speaking children (30), due to the unavailability of a certified Bosnic C-ACT version. Outcome of the questionnaire is expressed as symptom score with a value of 0 implying full asthma and a value of 25 representing no asthma symptoms.

Spirometry and FeNO

A Masterscope PC system from Viasys Healthcare GmbH (Viasys, Hoechberg, Germany) was used for spirometry following American Thoracic Society/European Respiratory Society (ATS/ERS) recommendations (31). Respiratory parameters analyzed were peak expiratory flow (PEF), forced expiratory volume in 1 s (FEV_1), percent forced expiratory volume in 1 s of forced vital capacity (FEV_1%FVC), forced expiratory flow at 25% exhaled forced vital capacity (FEF25), forced expiratory flow at 50% exhaled FVC (FEF50), and maximum mid-expiratory flow over the middle half of the FVC (MMEF25/75).

FeNO, a surrogate marker for eosinophilic inflammation was measured online according to the ATS and ERS

TABLE 1.—Study timeline.

Day	0	1	3	5	7	9	10	11	13	14	15	17	18	19	20	50	80	140
Arrival/departure	x														x			
Spirometry			x	x	x	x		x	x		x	x		x			x	
FeNO		x					x			x			x		x		x	
ACT		x						x						x		x	x	x
Blood collection		x													x			

Notes: The asthma camp lasted from day 0 to day 20. ACT, Asthma Control Test; FeNO, fractional exhaled nitric oxide.

guidelines (32). The single breath online maneuver was performed with the NIOX MINO® system from Aerocrine (Solna, Sweden), according to manufacturer's instructions. All lung function testings were performed by two trained scientists.

Blood and Serum Analysis

Venous blood (13 ml per child) was collected in three tubes (BD Vacutainer® System, Becton Dickinson AG, Vienna, Austria) for testing serum (serum separation tube (SST) II Advanced), whole blood potassium (K^2) ethylene diamine tetraacetic acid (EDTA) (K^2EDTA), plasma, and PBMC (cell preparation tube (CPT)), according to manufacturer's guidelines. Sera were prepared immediately by keeping the tubes at room temperature for 30 minutes to allow coagulation. After centrifugation at 2000 × *g*, the sera supernatant was stored at −20°C. EDTA and CPT tubes were transported within 2 hours for further preparation after the first blood collection.

IL-5, IL-10, and IL-13 enzyme-linked immunosorbent spot assays (ELISpot) (BD Becton Dickinson AG) were performed according to manufacturer's instructions. Briefly, 1×10^5 PBMC per well were cultured and stimulated with PMA (12-O-tetradecanoylphorbol-13-acetate) and ionomycin (Sigma Aldrich, Vienna, Austria) for 24 hours. ELISpot analysis was performed with ImmunoSpot® Software (C.T.L. Europe GmbH, 53225 Bonn, Germany) and eosinophil cationic protein (ECP) enzyme linked immunosorbent assay (ELISA) (MBL, Mo Bi Tec, 37083 Göttingen, Germany) was carried out according to the manufacturer's protocol. The calculation of human ECP was done by calibration using a standard curve based on reference standards.

Measurements of total sera IgE and the ImmunoCAP (CAP) classes of timothy grass, rye, birch, mugwort, *Cladosporium herbarum*, cat dander, dog dander, and *Dermatophagoides pteronyssinus* were performed via ImmunoCAP on a Phadia 100 System (Phadia, Uppsala, Sweden).

Flow cytometry (FACS) analysis of PBMC was performed with a BD FACSArray™ (Becton Dickinson AG (BD), Schwechat, Austria) using the following monoclonal antibodies: CD4-Alexa Fluor® (AF) 488, CD25-APC-Cy7, and CD127-AF647. Data analysis was performed by BD FACSDiva Flow Cytometry Software version 5.03 (BD, Schwechat Austria).

Gene Expression Analysis

RNA was isolated from EDTA whole blood using RiboPure™-Blood (Applied Biosystems, 2345 Brunn am Gebirge, Austria) according to manufacturer's guidelines. Reverse transcription was performed with Revert Aid H Minus M-muLV Reverse Transcriptase (Fermentas, Vienna, Austria) with oligo(dT) primers following the manufacturer's protocol.

Polymerase chain reaction (PCR) primers and corresponding TaqMan probes for IL-10, IL-13, interferon gamma (IFN-γ), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and RPL13a were designed by Microsynth AG (Balgach, Switzerland). The primer and probe sequences were as follows: IL-10 forward, 5′tacggcgctgtcatcgatt3′; IL-10 reverse, 5′ggcattcttcacctgctcca3′; IL-10 probe, 5′cttccctgtgaaaacaagagcaaggc3′; IL-13 forward, 5′cctggaatccctgatcaacg3′; IL-13 reverse, 5′cgctcagcatcctctgggtX3′; IL-13 probe, 5′tcaggctgcagtgccatcgagaa3′; IFN-γ forward, 5′ccaacgcaaagcaatacatga3′; IFN-γ reverse, 5′cgcttccctgttttagctgc3′; IFN-γ probe, 5′atccaagtgatggctgaactgtcgcc3′; GAPDH forward, 5′ctgttcgacagtcagccgc3′; GAPDH reverse, 5′ggtgtctgagcgatgtggc3′; GAPDH probe, 5′tcttcttttgcgtcgccagccg3′; RPL13a forward, 5′caagttgaagtacctggctttcca3′; RPL13a reverse, 5′tggcctcgcttggttttgtgc3′; RPL13a probe, 5′cctcgcacggtccgccagaagatg3′. cDNA was amplified by using Maxima™ Probe qPCR Master Mix (2×) (Fermentas) following the recommendations of the manufacturer in a Rotorgene 6000 HRM (Corbett, GenXpress, Wiener Neudorf, Austria). GAPDH was used as a housekeeping gene for normalization (33). Relative quantification and calculation of the range of confidence were performed by using the algorithms outlined by Vandesompele et al. (34). All amplifications were carried out in quadruplicates.

Statistical Analysis

All analyses were performed using IBM SPSS statistics version 20 (IBM, New York, USA, www.spss.com). For each parameter, the changes (delta) between study start (baseline) and all succeeding time points, as well as between the two groups at study end, were calculated. Shapiro–Wilk test was used for the distribution of these quantitative variables. Differences were compared by means using *t*-test for paired and unpaired variables with a normal distribution. For variables with a non-normal distribution, Wilcoxon signed-rank test or Mann–Whitney *U*-test was applied.

The baseline characteristics of the study participants are shown as medians, including 25th and 75th percentiles, with their respective *p*-values (Table 2). Figures showing C-ACT, FeNO, lung function, ELISpot, real-time PCR (RT-PCR), ECP Elisa, and FACS are shown as mean ± standard error of mean (SEM). Statistical significance was expressed as $p \leq .05$ (*) or as $p \leq .01$ (**).

RESULTS

Patient Characteristics

The randomized controlled clinical study comprised 54 children, including 40 children from Austria and 14 children from Bosnia/Herzegovina (18 females and 36 males) ranging from 8 to 15 (mean 11.0 ± 1.86 SD) years of age. We found no significant differences between the measured baseline values of both groups (Table 2).

Sensitization Pattern and Course of Blood IgE Levels

IgE levels greater than 90 kU/l could be detected in 48 out of 54 children at the beginning of the study (day 1), with an average total IgE level of 644.3 ± 175.6 kU/l (mean ± SEM) in the water aerosol group and 652.6 ± 158.6 kU/l (mean ±

TABLE 2.—Patients characteristics at study start.

Parameter	Control group $n = 27$	Water aerosol group $n = 27$	p-Value
Age (years)	11.0 (10.0–12.0)	11.0 (9.0–12.0)	.95
Female	8	10	–
Male	19	17	–
BMI	18.9 (16.1–22.2)	17.6 (16.3–21.1)	.46
FEV_1 (l)	2.3 (1.7–2.7)	2.2 (1.9–2.4)	.63
FEV_1 percentage of predicted	84.6 (80.0–96.7)	79.0 (74.5–87.4)	.63
ACT score	19.0 (14.8–20.8)	19.4 (17.0–23.1)	.38
Total IgE (kU/l)	230.5 (138.0–1070.0)	293.0 (153.0–768.0)	.9
HDM CAP class	3.0 (0.0–4.0)	4.0 (1.0–5.0)	.17

Notes: Median (25–75th percentile). HDM, house dust mite (*Dermatophagoides pteronyssinus*); ACT, Asthma Control Test; BMI, body mass index; FEV_1, forced expiratory volume in 1 s.

TABLE 3.—CAP classes for eight different allergens (mean ± SEM).

Allergen	Number of total	Average CAP class, day 1	Average CAP class, day 20
Timothy grass	35	3.9 ± 1.6	3.9 ± 1.5
Rye	34	3.7 ± 1.6	3.9 ± 1.5
Birch	27	3.7 ± 1.4	3.7 ± 1.6
Mugwort	18	2.3 ± 1.1	2.5 ± 1.0
Cladosporium herbarum	7	2.0 ± 1.2	2.0 ± 1.3
Cat dander	33	2.7 ± 1.2	2.7 ± 1.2
Dog dander	27	2.2 ± 0.8	2.2 ± 0.8
Dermatophagoides pteronyssinus	39	4.1 ± 1.5	4.1 ± 1.5

Notes: Average values only include positive individuals. SEM, standard error of mean.

SEM) in the control group. Total levels of IgE in all children did not change between day 1 and day 20 ($p = .317$).

Table 3 depicts the sensitization pattern and mean CAP class of patients at day 1 and day 20 of the study. Eight children were monosensitized, 46 were sensitized against more than one allergen, and 25 were sensitized against six or more allergens. Similar to total IgE levels, allergen-specific IgE levels did not significantly change between day 1 and day 20.

Environmental Parameters

Counting of pollen revealed moderate number/m^3, with two peaks in the middle of July (80 pollen/m^3). Clinically relevant pollens measured at the site of accommodation were grass pollen (peak at 38 pollen/m^3) and plantago pollen (peak at 11 pollen/m^3). More than 1000 mold spores/m^3 were measured on 6 days in July. No other clinically relevant pollen numbers were detected for the duration of the study.

The water aerosol group probands were exposed at a site between 55 and 65 m away from the base of the Krimml Waterfalls on the orographic right side. Wind velocity at this site varied in the range of 2–5 m/s. The concentration of the most abundant 2 nm negative ions ranged between 10×10^3 and 15×10^3 ions/cm^3, whereas the heavier aerosol ions (peak size 120 nm) were present in much smaller concentrations (200–300 ions/cm^3).

According to Henshaw et al. (35), the total deposition probability of inhaled aerosol particles in all compartments of the human lung is 100% for aerosol particles with diameters less than 10 nm. Assuming that healthy humans inhale about 6000 cm^3 of air per minute and that the average concentration of negative air ions at the waterfall exposure site is 12,500 ions/cm^3, with every single ion carrying a charge of 1.6×10^{-19} C, then the resulting delivered charge to the lung would amount to around 1.2×10^{-11} C per minute. According to these calculations, in 1 hour of exposure at the waterfall site the lungs of the patients would be exposed to about 7.2×10^{-10} C (i.e., 4.5×10^9 electrons).

The average concentration of positive ions at the waterfall site varied from 200 to 700 ions/cm^3. The concentration of ions at the site of the control group was within background daily limits, i.e., 400–600 ions/cm^3 of both polarities.

Analysis of the nano-aerosol size at the site of waterfall exposure revealed a 1.5–3 nm peak with maximum measurements of up to 175 particles/cm^3. This aerosol peak was found to be completely absent at the control exposure site (50 particles/cm^3).

Asthma Questionnaire

At the start of the sojourn, control group and water aerosol group probands had similar average symptom scores of 18.2 and 19.4, respectively, which were not significantly different between the two groups (Figure 2). At the end of the asthma camp, both groups showed a significant increase in the scores of the Asthma Control Test (ACT). In the follow-up phase, the change in ACT scores was significantly lower in the control group compared to the scores of the water aerosol group. The scores of the latter only marginally declined after the camp (between day 20 and day 140). At day 140 the ACT scores of the water aerosol group were still significantly elevated compared to the baseline assessment and the change of ACT scores was significantly different between the water aerosol group and the control group ($p = .047$) (Figure 2).

High Altitude and Waterfall Treatment Reduces Local Airway Inflammation and Improves Lung Function

FeNO values at study start were 42.6 ppb for the water aerosol group and 33.7 ppb for the control group ($p = .37$) (Figure 3). During the asthma camp, FeNO values

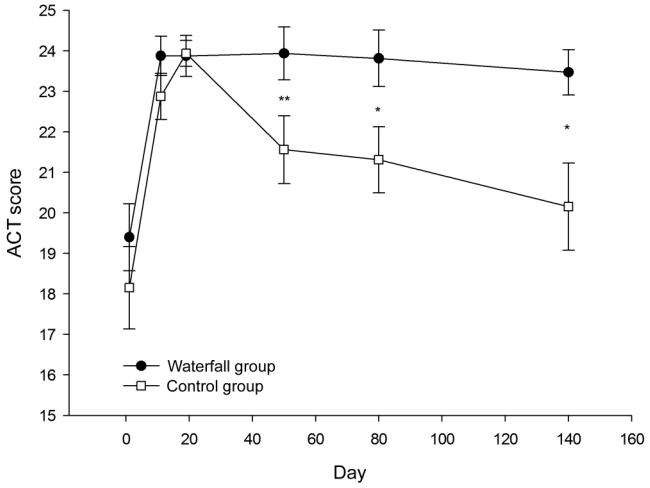

FIGURE 2.—Self-assessment of asthma control (C-ACT). Time course of the self-assessed asthma control test over a period of 4 months. The water aerosol group showed a persistent improvement with a significant difference ($p = .047$) in the mean of differences between study start (day 1) and study termination (day 140). Error bars show SEM. Asterisks show the intergroup comparison. *$p < .05$, **$p < .01$.

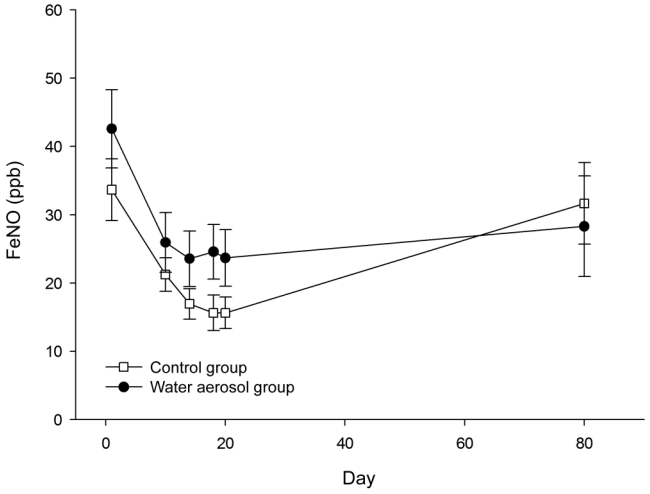

FIGURE 3.—Eosinophilic airway inflammation measured by FeNO. FeNO values significantly decrease in both groups during the asthma camp (day 1–day 20, $p < .001$), with a marked sustainability during the follow-up period in the water aerosol group ($p < .01$).

significantly decreased in both groups (water aerosol group from 42.6 to 23.7 ppb = 44.4%, $p < .001$; control group from 33.7 to 15.6 ppb = 53.6%, $p < .001$). The FeNO reduction proved to be sustainable in the water aerosol group (up to day 80 in the follow-up period), with a significant difference of −13 ppb compared to the start of the study ($p < .01$). In contrast, at the end of the follow-up period, FeNO values of the control group approached the values measured at the start of the study (–5 ppb, $p = .32$). Depicted as a percentage of the initial values, FeNO was reduced by 33.6% in the water aerosol group compared to only 6% in the control group (day 80). This constitutes a strong trend toward lower FeNO in the water aerosol group, which, however, did not reach statistical significance ($p = .08$) (Figure 3).

Measuring lung function using several respiratory parameters did not reveal differences between the groups at the start of the study and a positive effect on lung function could be detected in both groups during the whole period of the asthma camp (Figure 4). Interestingly, all measured parameters of lung function increased significantly in the water aerosol group, whereas only the increase in PEF proved to be statistically significant in the control group (Figure 4).

In the follow-up measurements at day 80, a trend towards a sustainable positive effect could be observed in the water aerosol group, with an increased PEF as compared to the control group ($p = .07$). With respect to the other lung function parameters, no differences were detectable between the groups 2 months after the end of the camp (data not shown).

Inflammatory and Anti-Inflammatory Cytokine Pattern, Cytokine Gene Expression, and Measurement of ECP

Analysis of cytokine production by PBMCs showed that in both the water aerosol group and the control group, the number of IL-5- and IL-13-producing cells declined between day 1 and day 20. The decrease in IL-13-producing cells was highly significant in both groups, whereas the decrease in IL-5-producing cells proved to be statistically significant only in the water aerosol group. Interestingly, a statistically significant increase in IL-10-producing cells was also only detected in the water aerosol group (Table 4). The frequencies of cytokine-producing cells though were not significantly different between the two treatment groups at day 20.

However, RT-PCR analysis of PBMCs at day 20 revealed a significantly lower IL-13 expression in cells from the water aerosol group compared to the control group, whereas IL-10 and IFN-γ expression were found to be increased in PBMCs from both groups (Table 4).

ECP measured in serum is derived from activated eosinophils and can be used as a surrogate marker for eosinophilia. Compared to initial measurements at day 1, reduced ECP concentrations were detected in both groups at day 20, with no significant changes in sera ECP concentrations between the two treatment groups (Table 4).

Therapeutic Effects on Treg Cells in the Blood

Treg cells were analyzed as a subset of lymphocytes according to the expression of the surface markers $CD4^+$ $CD25^+$ $CD127^{low}$ (36). At day 20, the number of Treg cells had increased significantly in both groups and there was no statistically significant difference between the water aerosol group and the control group (Table 4).

DISCUSSION

Traditionally, numerous beneficial health effects have been attributed to waterfalls in various regions of the world, including areas around waterfalls in Austria (37).

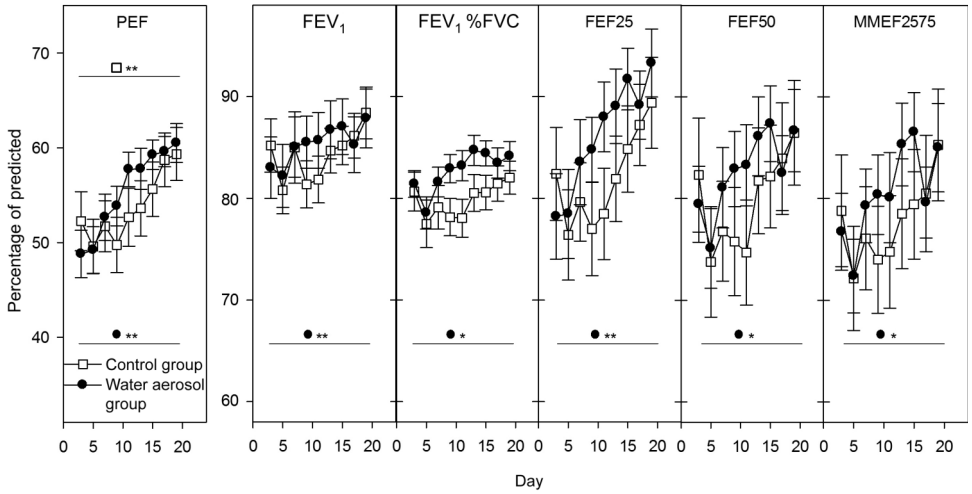

FIGURE 4.—Measurement of lung function parameters during the asthma camp. For both groups, all parameters indicate improvement in lung functions between day 1 and day 20, with a statistical significance concerning all parameters in the water aerosol group and a significant bettering of PEF in the control group. $*p \leq .05$, $**p \leq .01$. Error bars are shown as SEM.

TABLE 4.—Blood and serum parameters at day 1 (T1) and day 20 (T2).

Parameter	WAG T1 ± SEM	WAG T2 ± SEM	WAG delta (p-value)	CG T1 ± SEM	CG T2 ± SEM	CG delta (p-value)	Group difference (p-value)
IL-5 ELISpot (CFU)	94.6 ± 14.77	62.8 ± 8.1	−31.8 ($*p = .03$)	86.4 ± 14.1	70.5 ± 12.0	−15.9 ($p = .19$)	15.9 ($p = .38$)
IL-10 ELISpot (CFU)	31.8 ± 4.9	51.6 ± 9.7	19.8 ($*p = .04$)	35.6 ± 5.5	41.6 ± 5.8	6.0 ($p = .43$)	13.2 ($p = .26$)
IL-13 ELISpot (CFU)	45.9 ± 4.6	27.1 ± 2.1	−18.8 ($**p < .01$)	44.4 ± 5.3	27.0 ± 2.1	−17.4 ($**p < .01$)	1.4 ($p = .64$)
IL-10 qRT-PCR (expression)	1	1.6 ± 0.2	0.6	1	1.8 ± 0.2	0.8	0.2 ($p = .38$)
IL-13 qRT-PCR (expression)	1	0.7 ± 0.1	−0.3	1	1.1 ± 0.2	0.1	0.4 ($*p = .03$)
IFN-γ qRT-PCR (expression)	1	1.3 ± 0.2	0.3	1	1.3 ± 0.1	0.3	0.0 ($p = .78$)
ECP (ng/ml)	5.2 ± 0.4	4.2 ± 0.5	1.0 ($*p = .03$)	5.3 ± 0.6	4.6 ± 0.5	0.7 ($p = .10$)	0.3 ($p = .75$)
Treg cells (%)	4.0 ± 0.2	4.7 ± 0.2	0.7 ($**p < .01$)	4.0 ± 0.2	4.5 ± 0.2	0.5 ($*p = .01$)	0.2 ($p = .59$)

Notes: Statistical analysis was performed between day 1 and day 20 for the water aerosol group (WAG) and the control group (CG), as shown in columns WAG delta and CG delta. Intergroup comparison is demonstrated for the deltas of day 20 to day 1 (group difference). The baseline values of qRT-PCR were set to 1; therefore, only intergroup statistics were performed between the groups. SEM, standard error of mean; IL, interleukin; ECP, eosinophil cationic protein; Treg cells, regulatory T cells; ELISpot, enzyme-linked immunosorbent spot assay; CFU, colony-forming unit; qRT-PCR, quantitative real-time PCR; IFN-γ, interferon gamma.
P-values: $*p \leq .05$, $**p \leq .01$.

In most cases, folk memory describes mitigating effects concerning respiratory diseases.

Indeed, recent physical investigations have revealed a specific environment around waterfalls. Break-up of small water droplets in the waterfall forms specific nano-aerosols comprising mainly negatively charged intermediate ions, which are assumed to trigger a variety of biological effects (25, 38, 39).

With the present randomized controlled pediatric clinical study, we addressed the question of whether the specific environment of a waterfall provides additional beneficial effects on pediatric allergic asthma compared to climate therapy alone. To this end, two groups of children were treated identically during a 3-week "Asthma Camp", except for one group being exposed to water aerosol for 1 hour every day.

In general, the results of our study are in agreement with publications indicating health benefits of high-altitude climate therapy such as the mitigation of asthma symptoms and triggering of immunomodulatory effects (12–14). Our data clearly demonstrate that lung function parameters and the allergic inflammatory status of the patients were positively influenced by the 3-week stay in the "Asthma Camp." In both groups, FeNO values decreased; the lung function parameters such as PEF, FEV_1, FEV_1%FVC, FEF25, FEF50, and MMEF25/75 increased; the number of IL-5- and IL-13-producing cells decreased; and the number of Treg cells increased during the asthma camp. Furthermore, self-assessment of asthma control revealed a significant improvement in the C-ACT score after the 3-week long camp.

In addition to the positive effects of general climate therapy, the daily 1-hour exposure to the waterfall environment caused additional benefits. A clear trend toward a synergistic effect of water aerosols could be measured concerning several parameters such as significantly reduced expression of IL-13, which is an asthma key cytokine, involved in the regulation of IgE synthesis, eosinophil infiltration, mucus hypersecretion, and subepithelial fibrosis (40). Furthermore, several molecular and functional asthma parameters showed more pronounced and significant changes (day 1/day 20) in the water aerosol

group compared to the control group, such as reduced levels of IL-5-producing cells, enhanced frequencies of IL-10-producing cells, and lower ECP as well as an increase in most lung function parameters.

More importantly, water aerosol exposure combined with high-altitude therapy improved the sustainability of the positive effects. In the follow-up period (day 80), PEF was 15% above baseline in the water aerosol group and 2% above baseline in the control group ($p = .026$, data not shown). A similar trend could be detected for all other lung function parameters, with increased mean values in the water aerosol group in comparison to the control group values, which had returned to baseline at this point. Additionally, the results of FeNO measurements at day 80 indicated a long-lasting positive effect of water aerosols, as FeNO was reduced by 33% in the water aerosol group compared to only 6% in the control group.

In confirmation with the results of the molecular and functional assays, the C-ACT scores were higher in the water aerosol group compared to the control group in the follow-up period (up to 140 days, $p = .047$). Nevertheless, 4 months after the camp both groups still showed ACT scores above 20, thus indicating good asthma control.

At present, neither the mechanisms underlying climate therapy nor those underlying the influence of water aerosols are known. Our results point to a synergistic effect of the latter, especially concerning the sustainability of the treatment effects. However, the data are partly limited by the fact that no validated Bosnian translation of the C-ACT was available at the time of our study, thereby limiting our survey to the German-speaking subpopulation. Furthermore, we cannot exclude that individual personal strategies of coping with asthma, utilized by the patients after the end of the camp, might have influenced the results.

In summary, our data confirm the general hypothesis that high-altitude climate therapy may enhance immunoregulatory pathways, which in turn reduce inflammatory responses (13), as demonstrated by an improvement in lung function, a reduction in the TH2-inflammatory parameters, and a concomitant increase in Treg cells and their key cytokine IL-10.

Moreover, our results also show a clear trend toward a synergistic effect of inhaling ionized water aerosol during the 1-hour stay close to the waterfall when compared to the effect of climate therapy alone.

In conclusion, we suggest that high-altitude climate therapy combined with exposure to ionized water aerosol has a beneficial effect on pediatric asthma. After 3 weeks of therapy, FeNO was reduced by about 50%, ECP was lowered by around 18%, and the children had only marginal asthma symptoms at the end of the camp. More importantly, the improved asthma control lasted for at least 4 months, emphasizing the potential role of climate and water aerosol therapy as a cost-effective and long-lasting treatment option for pediatric asthma.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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