

Ph.D. Thesis

**HISTAMINE IN ALLERGIC DISEASES:
AN OLD MOLECULE IN NEW CONCEPTS**

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LIST OF PUBLICATIONS

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II. Mitchell D, Paniker L, Sanchez G, Bella Z, **Garaczi E**, Szell M, Hamid Q, Kemeny L, Koreck A. Molecular response of nasal mucosa to therapeutic exposure to broad-band ultraviolet radiation.

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International Patent Applications

Garaczi E, Sugiyama H, Gyulai R, Cooper KD, McCormick TS.

Methods and reagents for identifying/isolating T regulatory (TREG) cells and for treating individuals.

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Bata Zs, **Garaczi E**, Gruber L, Hamar P, Kemény L, Kökény G, Lisiewicz J, Lőrincz O, Molnár M, Mózes M, Ötvös L, Pandúr J, Pintér I, Somogyi E, Szabó KA, Szollár L, Tőke E. Immunogenic nanomedicine composition and preparation and uses thereof.

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TABLE OF CONTENTS

ABBREVIATIONS.....	4
1. INTRODUCTION.....	5
2. AIMS.....	8
3. HISTAMINE IN CONTACT HYPERSENSITIVITY.....	9
3.1 Introduction.....	9
3.2. Materials and Methods.....	12
3.3. Results.....	15
3.4. Discussion.....	22
4. INTRANASAL PHOTOTHERAPY IN SEASONAL ALLERGIC RHINITIS.....	25
4.1. Introduction.....	25
4.2. Materials and Methods.....	26
4.3. Results.....	27
4.4. Discussion.....	30
5. CONCLUSION.....	31
6. SUMMARY.....	34
7. REFERENCES.....	35
ACKNOWLEDGEMENTS.....	40
APPENDIX	

ABBREVIATIONS

AC	acetone
ACD	allergic contact dermatitis
AHR	airway hyper-responsiveness
AR	allergic rhinitis
ARIA	Allergic Rhinitis and its Impact on Asthma
CHS	contact hypersensitivity
DNFB	2,4-dinitrofluorobenzene
DLN	skin-draining lymph nodes
DTH	delayed-type hypersensitivity
EAE	experimental autoimmune encephalomyelitis
HDC	histidine decarboxylase
H1R	histamine receptor type 1
H2R	histamine receptor type 2
Ig	immunoglobulin
RT-PCR	reverse transcription polymerase chain reaction
SAR	seasonal allergic rhinitis

1. INTRODUCTION

Histamine has been established to play a pathophysiological regulatory role in various immunological reactions. The main functions of histamine include H1 receptor (H1R) mediated actions on smooth muscle, vascular permeability and modulation of allergic response, and gastric acid secretion basically via H2 receptors (H2R). H3 receptor (H3R) is expressed in the nervous system, where it serves as a presynaptic feedback receptor on histaminergic neurons. H4 receptor (H4R), the last receptor discovered, is largely expressed in haemopoietic cells and its chemotactic properties designate its regulatory role in immunomodulation (1). Highly selective blockers and agonists suitable for *in vitro* and *in vivo* use have been developed for these histamine receptors. These include clinically established H1R and H2R blockers, as well as novel investigational drugs directed toward H4R (2).

Histamine is a diamine derivate of histidine that is produced under the control of a single enzyme, histidine decarboxylase (HDC). HDC deficient (HDC^{-/-}) mice were generated by Ohtsu and his co-workers about a decade ago (3). Destruction of the HDC gene results in a marked reduction of the tissue histamine content. These HDC^{-/-} mice represent a suitable experimental model to assess the role of histamine in allergic diseases.

In our present work, we investigated the immunoregulatory role of histamine using HDC^{-/-} mice in a highly prevalent allergic disease, in contact dermatitis. The central role of histamine in the pathophysiology of allergic rhinitis is well established. In the second part of this work we compared the efficacy of a H1R antagonist with a newly developed phototherapeutic device in patients with allergic rhinitis.

1.1. Contact dermatitis

The prevalence of allergic diseases is increasing in most countries of the world. The World Allergy Organization (WAO) reported the results of the survey on the prevalence of allergic rhinitis, asthma, atopic eczema, food allergy, dug allergy and hymenoptera hypersensitivity in 2007 (4). Based on the data provided by the WAO member societies in 2005, the prevalence rate of allergic rhinitis is above 16% in most countries, Hungary reported 17%. The prevalence of allergic asthma and atopic dermatitis was estimated between 6-15%.

Contact dermatitis is one of the most common occupational diseases (5). Two main types of contact dermatitis may be distinguished, according to the pathophysiological

mechanisms involved: irritant contact dermatitis and allergic contact dermatitis. Allergic contact dermatitis, also referred as contact hypersensitivity (CHS), requires the activation of antigen-specific acquired immunity.

Allergic contact dermatitis (ACD) is one of the most common dermatoses, and its socioeconomic impact as an acquired, job-related disease is enormous (6). ACD can produce an acute eczematous picture with erythema, vesicles and weeping and chronic eczema with hyperkeratosis, fissuring and lichenification (7). Diagnosis of ACD is usually confirmed by epicutaneous patch testing.

Contact allergens are low molecular weight, lipid-soluble chemicals that behave as haptens as they are not immunogenic by themselves. Haptens comprise a group of very diverse chemicals, including a limited number of strong contact sensitizers and thousands of weak haptens responsible for human ACD. Knowledge on the pathophysiology of ACD have been obtained from animal models, where strong experimental contact sensitizers [2,4-dinitrofluorobenzene (DNFB), dinitrochlorobenzene (DNCB), oxazolone] were used. These haptens are not present in our daily environment.

ACD is a classical delayed-type hypersensitivity reaction, or a type IV immunological response, that occurs in two phases: initially a sensitization that lasts for 10-15 days in humans, and 5-7 days in mice. The second phase is the elicitation (effector) response takes 24-72 hours after the reexposure of the same allergen. The early phase of the elicitation occurs 1-3 hours after antigen challenge in mice. This early phase has been postulated to depend on local tissue mast cells, the main source of histamine in the skin.

In the first part of this work we investigated the role of histamine in contact hypersensitivity reaction using histidine decarboxylase deficient (HDC^{-/-}) mice.

1.2. Allergic rhinitis

Allergic rhinitis (AR) is a common inflammatory disease that causes major illness and disability worldwide. The prevalence of AR was found to be around 25% in a study on the general population in Europe (8,9).

Patients with allergic rhinitis suffer from physical symptoms (sneezing, rhinorrhea, nasal itching and nasal obstruction) and from nonphysical symptoms including sleeping disturbance, emotional problems, impairment of activities and social functioning (10). It is associated with impairments in how patients function in day-to-day life. Impairment of

quality of life (QOL) is seen in adults and in children. The classical symptoms interfere with sleep, leisure, school or work activities.

According to the guidelines issued by the Allergic Rhinitis and its Impact on Asthma (ARIA) group, allergic rhinitis is subdivided to intermittent (IAR) and persistent (PER), based on the number of consecutive days when patients experience symptoms (10). Previously, AR was subdivided based on the time of exposure into seasonal, perennial and occupational. Seasonal allergic rhinitis is related to a wide variety of outdoor allergens such as pollens or molds.

Allergic rhinitis is an immunoglobulin E (IgE)-mediated inflammatory disease of the nasal mucosa. The central role of histamine in the pathomechanism of AR is well established (11). Specific IgE elicits hypersensitivity reaction through binding FcεRI receptors on mast cells. Their main preformed mediator is histamine which induces vasodilatation, increased vessel permeability, edema and mucous hypersecretion in the mucosa, thereby causing clinical symptoms. The ARIA guideline recommends using second generation antihistamines as first-line treatment for AR. However, the treatment of allergic rhinitis is not always satisfactory and some patients fail to respond to treatment.

It has been demonstrated that selective H1R antagonists failed to completely inhibit nasal allergic reaction in mice, suggesting the involvement of other receptors in these responses (12). Supporting results were obtained from H1R knockout mice. Thus, it is considered that histamine receptor blocking alone is not sufficient for complete understanding of the mechanisms of allergic reactions in animals.

In the second part of our work we compared the efficacy of a second generation antihistamine with that of a recently developed new phototherapeutic modality in patients with seasonal allergic rhinitis.

2. AIMS

2.1. To investigate the role of histamine in contact hypersensitivity

1. We compared the CHS response in HDC^{-/-} mice with that of in wild type mice.
2. We measured the cell composition of the axillary and inguinal lymph nodes.
3. We measured the composition of infiltrating cells in the ear skin.
4. We measured the expression of IL-2, IFN- γ , TNF- α and IL-4 genes in the ear skin.

2.2. To compare the efficacy of fexofenadine HCl, a second generation antihistamine with that of a new intranasal phototherapeutic device in patients with seasonal allergic rhinitis

1. A randomized open study was conducted in patients with a history of at least 2 years of moderate-to-severe ragweed-induced allergic rhinitis.
2. Thirty-one patients were randomly assigned to receive either intranasal phototherapy or fexofenadine HCl for 2 weeks.
3. Each patient kept a daily diary of symptoms. Total nasal score (TNS), a sum of scores for nasal symptoms (nasal obstruction, itching, rhinorrhea and sneezing) was also calculated.

3. HISTAMINE IN CONTACT HYPERSENSITIVITY

3.1. INTRODUCTION

Histamine is present in all tissues of the mammalian body and plays an important role in many physiological and pathological functions. The importance of histamine has been demonstrated in gastric acid secretion, contraction of smooth muscle, neurotransmission, wound repair, embryogenesis, hematopoiesis, allergic skin reaction and malignant growth (3, 13).

Histamine, synthesized by histidine decarboxylase, is produced mainly in mast cells, basophils and histaminergic neurons, but macrophages, dendritic cells and T lymphocytes also synthesize histamine (14–16). The production and release of histamine are modulated by various cytokines such as IL-1, IL-3, IL-5 and IL-8 (17). Histamine plays a regulatory role in Th1/Th2 balance at multiple points; however, the majority of histamine actions seem to promote Th2 responses (13, 18).

Four different membrane receptors of histamine (H1R, H2R, H3R and H4R) have been characterized pharmacologically and at the molecular level. One or more of these receptor types are expressed on many different cell types, including T cells, B cells, monocytes and neutrophils (13, 19). The secretion of IL-2 and IFN- γ from Th1 cells can be either inhibited or stimulated by histamine, and both effects are mediated *via* H2R receptors (17). It has been recently published that H1R is overexpressed on Th1 cells, while H2R is overexpressed on Th2 cells. H1R-deficient mice demonstrate suppression of Th1 cytokines and dominant secretion of Th2 cytokines. H2R-deficient mice show a significant enhancement of both Th1- and Th2-type cytokine secretion (20).

Contact hypersensitivity response develops in two distinct phases: sensitization and elicitation. In the sensitization phase, mice exposed to contact allergen showed an increase in the percentage of antigen (Ag) specific Thy1⁺/CD5⁺/CD3⁻/TCR⁻/B220⁺ cells in the skin-draining lymph nodes (DLN) (21, 22) (Figure 1). These B220⁺ (CD45R⁺) B cells produce IgM/IgG type antibodies that pass into the circulation and the extravascular tissues. These antibodies bind to receptors on the surface of mast cells and platelets and play a role in the increase of vascular permeability. Cytokines produced by Tc1 cells (IFN- γ), Th1 cells (IL-2, IFN- γ and TNF- α), Th2 cells (IL-4 and IL-10) and Langerhans cells (IL-12 and IL-18) are important for the optimal induction and initiation of CHS in DLN (23–25).

The elicitation phase is characterized by two distinct phases (Figure 1). In the early phase of elicitation, the antigen bound by IgM/IgG type antibodies produced by B220⁺ B cells leads to mast cell and platelet activation. Release of serotonin and TNF- α from these cells results in an increased vascular permeability (26–28). Geba *et al* found that delayed type hypersensitivity reaction (DTH) was either intact or only partially decreased in mast-cell deficient mice (29), and severe depletion of platelets with anti-platelet antibody strongly inhibited the contact hypersensitivity, especially in mast-cell deficient mice (30). These data suggest that serotonin and TNF- α are important mediators in the early phase of DTH.

In the later phase of elicitation (48-72 hours after challenge), antigen-specific T cells ($\alpha\beta$ T cells) are activated, resulting in the production of various cytokines. It is known that in the CHS reaction the main effector cells are IFN- γ -producing CD8⁺ Tc1 cells (6, 31, 32). The CHS responses are also regulated by IL-2, IFN- γ and TNF- α -producing CD4⁺ Th1 cells, as well as by IL-4 and IL-10-producing CD4⁺ Th2 cells (24, 31, 33–36)

Belsito *et al* reported, that H2R antagonist cimetidine augmented the CHS reaction by inhibition of the induction of T-suppressor cells (37). In contrast to this, the histamine H1R antagonist diphenhydramine, had no effect on suppressor cell activity in the CHS reaction in mice (38), and H1R antagonists did not cause the downregulation of CHS. Grob *et al* tested the effect of a prolonged treatment with H1R antagonist cetirizine on the reaction to a contact allergen applied by patch testing in a sensitized population (39). Their results demonstrated that the clinical recording did not show any difference between the cetirizine-treated and the control groups. These data suggest that histamine might contribute to the regulation of CHS through H2R receptor.

In the present study, we examined the CHS response in HDC knockout (HDC^{-/-}) histamine deficient mice. These mice were generated using a gene targeting method by Ohtsu *et al* (3). HDC^{-/-} mice exhibit a decreased number of mast cells. The lack of histamine leads to a large reduction in the overall contents of mast cell secretory granules, including proteases MMCP4, MMCP5 (chymases) and MMCP6 (tryptase) (40). In HDC^{-/-} mice, plasma extravasation could not be observed after passive cutaneous anaphylaxis test (41), suggesting that histamine plays a significant role not only in the anaphylactic increase of vascular permeability but also in the negative regulation of neutrophil infiltration (18).

The purpose of the present study was to determine the immunoregulatory role of histamine in dinitrofluorobenzene (DNFB)-induced delayed type hypersensitivity. We found, that the lack of histamine caused an intense Th1 type response, suggesting that histamine plays a negative regulatory role in contact dermatitis.

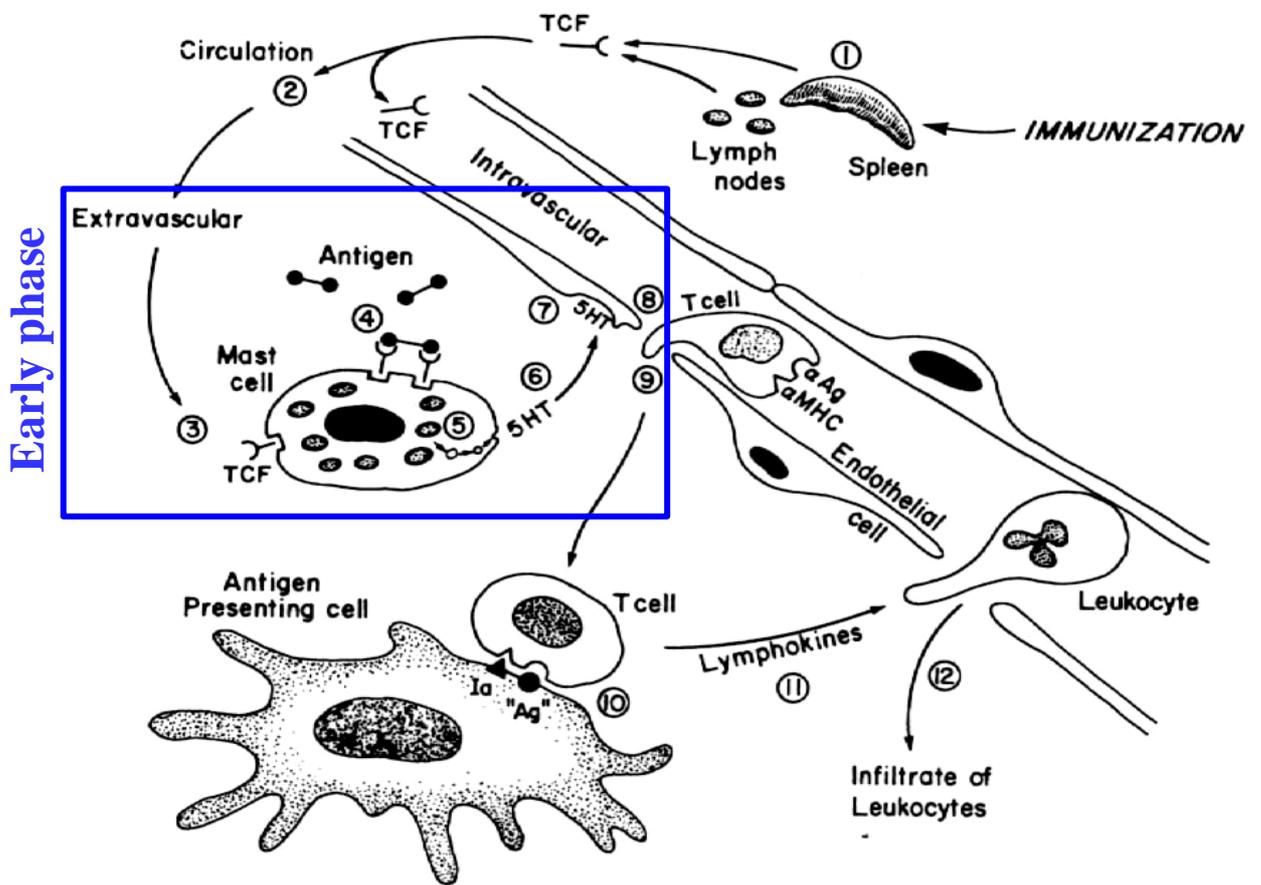


Figure 1. Hypothetical delayed type hypersensitivity (DTH) cascade in murine T cell-dependent skin reaction. From Askenase P.W. *Allergy. Principles & Practice* **1998**; 325 (21). TCF: Antigen-specific factors; 5-HT: serotonin.

3.2. MATERIALS AND METHODS

Animals

Generation of HDC^{-/-} mice was previously described (3). Female, 8-10-month-old HDC^{-/-} and CD1 background wild type mice were used in the experiments. Each experimental group consisted of 4-6 mice. The mice were kept on normal diet.

It was previously published an impaired reproduction of histamine deficient mice (42). Using CD1 background mice, the segregating F2 population contains a higher percentage of wild type mice (>25%) than HDC^{-/-} mice (<25%) (proportions are non-Mendelian) (unpublished data). Therefore, the HDC^{-/-} mice were randomly selected from F2 mice of the transgenic colony.

Treatment

The abdominal skin of the mice was shaved and sensitized with 25 µl 0.5% 2,4-dinitrofluorobenzene (DNFB; Sigma-Aldrich Corporation, St. Louis, MO, USA) in acetone/olive oil (4/1) for 2 consecutive days (days 0 and 1). Five days later, the dorsal surface of both ears was challenged with 15 µl 0.2% DNFB (n=6). The control mice were also sensitized with DNFB, but their ears were treated with acetone/olive oil (n=4). Ear thickness was measured with a spring-loaded micrometer (Oditest; Germany) before challenge and 24 and 48 hours after challenge. Treated ears were harvested 24 and 48 hours after the final application of DNFB or acetone/olive oil.

Flow cytometry

The axillary and inguinal lymph nodes draining the abdominal skin (sensitization area) were excised from each mouse 48 hours following challenge. For phenotypic analysis by flow cytometry, individual cell suspensions were prepared in Dulbecco's phosphate-buffered saline (PBS) with 5% fetal calf serum (GIBCO BRL, Paisley, Scotland) and 0.1% sodium azide (Merck, Darmstadt, Germany) (PBS-FCS) at 4°C and washed by centrifugation at 350 x g. The pellets were resuspended and diluted to 10⁷ cells/ml in PBS with 1% bovine serum albumin (BSA, fraction V; Sigma-Aldrich Corporation, St. Louis, MO, USA) and 0.1% sodium azide (PBS-BSA). Cells were labeled with the following rat anti-mouse monoclonal antibodies: anti-CD45 (M1/89 clone), anti-CD3 (KT3 clone), anti-CD4 (H129.19 clone), anti-

CD8 (53.6.72 clone), anti-CD45R (RA3-3A1 clone), anti-CD11b (M1/70 clone), anti-macrophage (F4/80 clone), anti-Gr-1 (RB6-8C5 clone). KT3, H129.19 and RB6-8C5 hybridomas were kindly provided by Professor W. Van Ewijk (Department of Immunology, Erasmus University, Rotterdam, The Netherlands), the other hybridomas were purchased from the American Tissue Type Collection (Rockville, MD, USA). Tissue culture supernatants were produced by culturing hybridomas in RPMI 1640 with 2mM L-glutamine, 10 mM Hepes, 2 g/l NaHCO₃, 10% FCS (GIBCO BRL, Paisley, Scotland) and 5 x 10⁻⁵ M 2-mercaptoethanol (Sigma-Aldrich Corporation, St. Louis, MO, USA). Twenty-five µl of cell suspensions was admixed to 25 µl samples of undiluted tissue culture supernatants in the wells of round-bottom microtiter plates. The plates were incubated at 4°C for 30 min, then the cells were washed three times with 200 µl/well PBS-FCS. The pellets were resuspended and incubated in 50 µl fluorescein isothiocyanate (FITC)-conjugated goat anti-rat Ig (Sigma-Aldrich Corporation, St. Louis, MO, USA) diluted to 1:200 at 4°C for 30 min. To avoid the cross-reactive binding of anti-rat Ig to mouse cell surface Ig, 2% normal mouse serum was admixed to the diluted anti-rat Ig. After washing, the cells were resuspended in 200 µl PBS-BSA and the dead cells were stained by adding 10 µl of 25 µg/ml propidium iodide (Sigma-Aldrich Corporation, St. Louis). 10⁴ cells per sample were analysed with a FACStar^{Plus} (with an argon ion laser, wavelength 488 nm; Becton Dickinson, Sunnyvale, CA, USA). The data were analyzed and the percentages of positive cells were calculated with the Cell Quest 3.1F software (Becton Dickinson) (43).

Histology

Ear samples were taken 24 and 48 h after DNFB painting and fixed in 4% formalin for routine histology with hematoxylin-eosin and toluidine blue staining. The sections were examined with an objective of 40x magnification.

Immunohistochemistry

Fresh frozen skin specimens were embedded in cryomatrix (Shandon, Life Sciences International, U.K.), 3 µm serial cryostat sections were prepared and avidin-biotin-peroxidase complex (ABC) method was used for immunohistologic staining. The sections were air dried, acetone fixed, then incubated with 0.5% BSA (Sigma-Aldrich Corporation, St. Louis, MO, USA) before adding the primary antibodies (Pharmingen, Becton Dickinson Company): rat anti-mouse CD45 and rat anti-mouse CD3 monoclonal antibody. Normal rat serum (DAKO,

Denmark) was used as negative control. The sections were incubated with biotin-conjugated rabbit anti-rat IgG (Vector Laboratories, Inc. Burlingame, CA), then with avidin-biotin peroxidase (Vectastain Elite kit, Vector Laboratories, Burlingame, CA). The peroxidase reaction was developed with 3-amino-9-ethylcarbazol (AEC; Sigma-Aldrich Corporation, St. Louis, MO, USA) and the sections were counterstained with hematoxylin.

Real-time reverse transcription polymerase chain reaction (Real-time RT-PCR)

Ear specimens taken 24 and 48h after the DNFB treatment were homogenized in Trizol reagent (Life Technologies) and total RNA was isolated following the instructions of the users' manual. RNA concentration was determined by the A260 value of the samples. First strand cDNA was synthesized from 3 µg total RNA in a 20 µl final volume by using a First Strand cDNA Synthesis Kit (MBI Fermentas, Vilnius, Lithuania). After reverse transcription, real-time RT-PCR was used to quantify the relative abundance of products of each genes (iCycler IQ Real Time PCR, Biorad) using primers specific for mouse GAPDH, IL-2, IL-4, IFN-γ and TNF-α. 2 µl aliquots of the reverse transcription volume were used as templates for PCR reactions.

The sequences for primers specific for IL-2 , IL-4, IFN-γ, TNF-α, GAPDH are shown in the attached scientific paper. The conditions of the reactions were as follows: 95°C for 5 min followed by 40 cycles at 95°C for 15 s (denaturation), and at 57°C for 45 s (annealing and elongation). The Mg²⁺ concentration was 3 mM, the concentration of the primers was 300 mM. Real time detection of PCR products was carried out by using SYBR Green I dye. Relative gene expression was calculated using the $\Delta\Delta C_t$ method.

Statistical Analysis

Student's *t* test was used for statistical evaluation, $P < 0.05$ was considered as significant.

3.3. RESULTS

HDC^{-/-} mice demonstrated increased contact hypersensitivity to DNFB

HDC^{-/-} and wild type mice were sensitized with 0.5% DNFB for 2 consecutive days. Five days later, the dorsal surface of the ears was challenged with 0.2% DNFB or with the solvent (acetone/olive oil). Ear thickness was measured before challenge, 24 and 48 hours after challenge. Twenty-four hours after challenge the DNFB induced increase of the ear thickness was significantly higher in the HDC^{-/-} mice (n=6) than in wild type mice (n=6) (mean±SD: $9.83 \pm 3.9 \times 10^{-2}$ mm vs. $6.3 \pm 2.8 \times 10^{-2}$ mm, $P < 0.05$) (Figure 2). Forty-eight hours after challenge, the ear thickness was still higher in HDC^{-/-} mice compared to wild type mice, but the difference was not significant between the two groups (mean±SD: $12.4 \pm 3.3 \times 10^{-2}$ mm vs. $7.2 \pm 5.2 \times 10^{-2}$ mm, $P > 0.05$) (Figure 2).

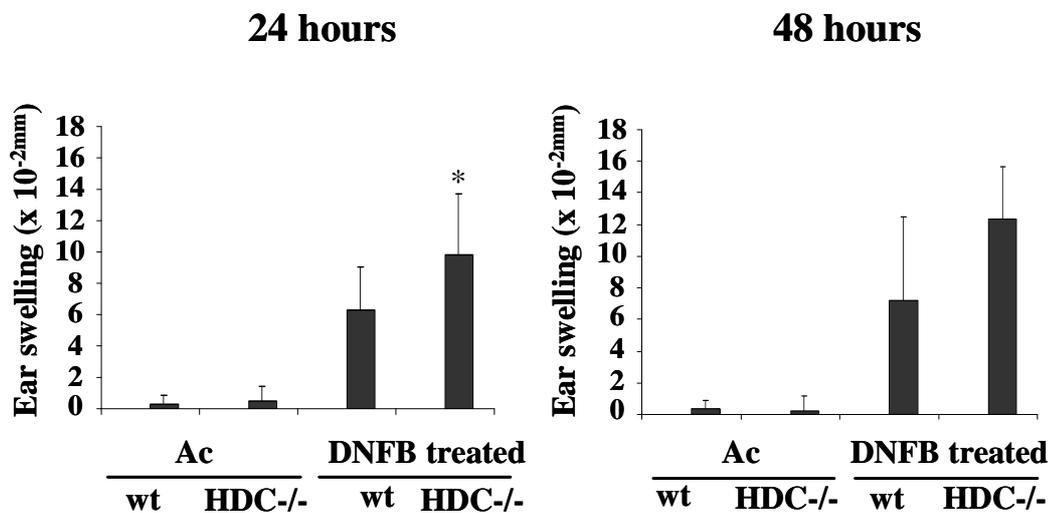


Figure 2. DNFB induced increase of ear thickness in HDC^{-/-} and wild type mice. Contact hypersensitivity response was challenged with 0.2% DNFB in sensitized HDC^{-/-} (n=6) and wild type mice (n=6). The control mice were also sensitized with DNFB, but their ears were treated with acetone/olive oil in both groups (n=4). Ear swelling was measured 24 (A) and 48 (B) hours after challenge with a micrometer. Data are presented as the mean ± SD. * $P = 0.023$ DNFB treated HDC^{-/-} vs. DNFB-treated wild type mice.

The percentages of CD4⁺ Th and CD8⁺ Tc cells were lower, those of CD45R⁺ B cells were higher in the DLNs of HDC^{-/-} mice

The axillary and inguinal DLNs were excised 48 hours after the DNFB challenge and cell suspensions were prepared for phenotypic analysis by flow cytometry. No significant difference was observed between the total number of the DLN cells in the DNFB treated HDC^{-/-} (mean±SD: 49.54± 15.87 x10⁶ vs. 43.25± 7.11x10⁶, *P*>0.05) and wild type mice. The percentages of CD3⁺ T (45.4±3.9% vs. 61.2%±4.1%), CD4⁺ Th (37.7±3.9% vs. 44.6±2.5%) and CD8⁺ Tc (11.7±1.7% vs. 19.2±3.4%) were significantly lower in the HDC^{-/-} mice. In contrast, the percentage of CD45R⁺ B cells (39.8±5.1% vs. 28.5±6.4%) was significantly higher in the HDC^{-/-} mice than in the wild type mice (Figure 3). The percentages of granulocytes (6.1±1.7% vs. 6.3±1.3%) and macrophages (2.1±1.7% vs. 1.4±1.0%) did not differ in the two groups. Similar differences were seen in the cell composition of the axillary and inguinal lymph nodes of untreated HDC^{-/-} and wild type mice. The percentages of the different cell subpopulations did not differ significantly from those found in the appropriate DNFB treated groups (data not shown).

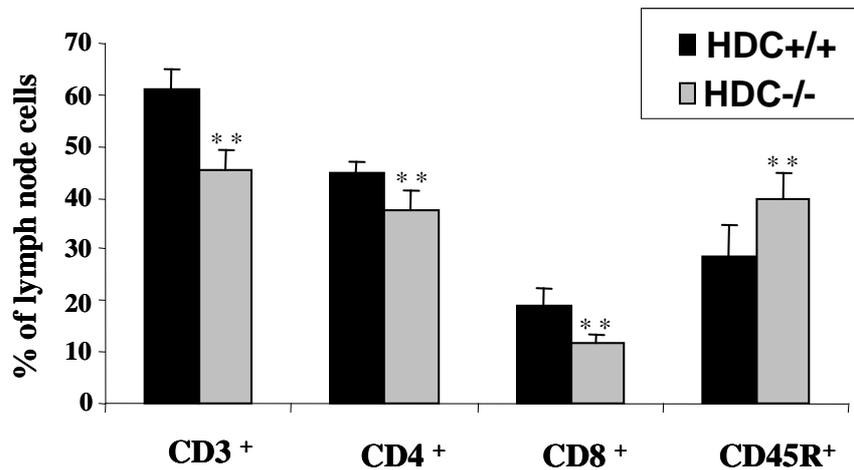


Figure 3. Distribution of lymphocyte subpopulations in DLN. The DLN were excised 48 hours following the DNFB challenge and analysed by flow cytometry. ** *P*<0.001 DNFB sensitized HDC^{-/-} (n=6) vs. DNFB sensitized wild type mice (n=6).

The number of infiltrating cells was higher in the ear specimens of HDC^{-/-} mice

Histologic sections were made from the ears 24 and 48 hours after challenge. In contrast to the acetone/olive-treated ear specimens (n=4), in the DNFB-painted ears of both HDC^{-/-} (n=6) and wild type mice (n=6) a cellular infiltrate and edema was seen. The majority of the infiltrating cells were neutrophil granulocytes and mononuclear cells in both DNFB treated groups at 24 and 48 hours after challenge, but the number of infiltrating cells and the degree of edema was higher in the HDC^{-/-} mice (Figure 4). In ear samples taken 24 or 48 hours after DNFB painting, mast cells were stained with toluidine blue. At these time points no difference was detected in the number of mast cells in the histologic sections of DNFB challenged and acetone/olive treated ears of either HDC^{-/-} or in wild type mice (data not shown).

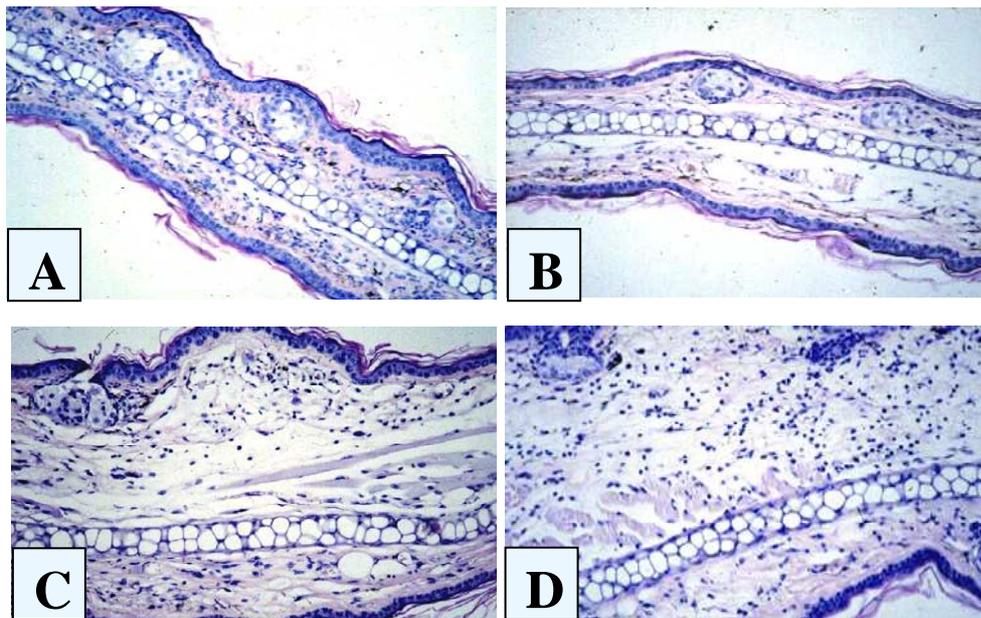


Figure 4. Hematoxylin eosin staining of DNFB-treated ears 24 hours after challenge. No inflammation was observed in the ears of DNFB-sensitized wild type (A) and HDC^{-/-} mice (B) following acetone/olive oil treatment (n=4). Neutrophil granulocytes and macrophages were the dominant cell types in the dermis 24 hours after DNFB challenge both in the HDC^{-/-} (D) and in the wild type mice (C) (n=6). The degree of edema and the number of infiltrating cells were higher in the HDC^{-/-} mice (D) compared to the wild type mice (C).

Strong CD45⁺ leukocyte infiltration was observed in the ears of HDC^{-/-} mice

In order to characterize the phenotype of the infiltrating cells, 3 μm cryostat sections were prepared and the ABC method was used for the immunohistologic staining. We observed a significantly higher percentage of CD45⁺ leukocytes in the dermis of the ears of the HDC^{-/-} mice (n=6) than in that of wild type mice (n=6). The number of CD3⁺ T cells was not increased in the DNFB-painted ears compared to the control (acetone/olive-treated) ones (n=4) in either group (Figure 5).

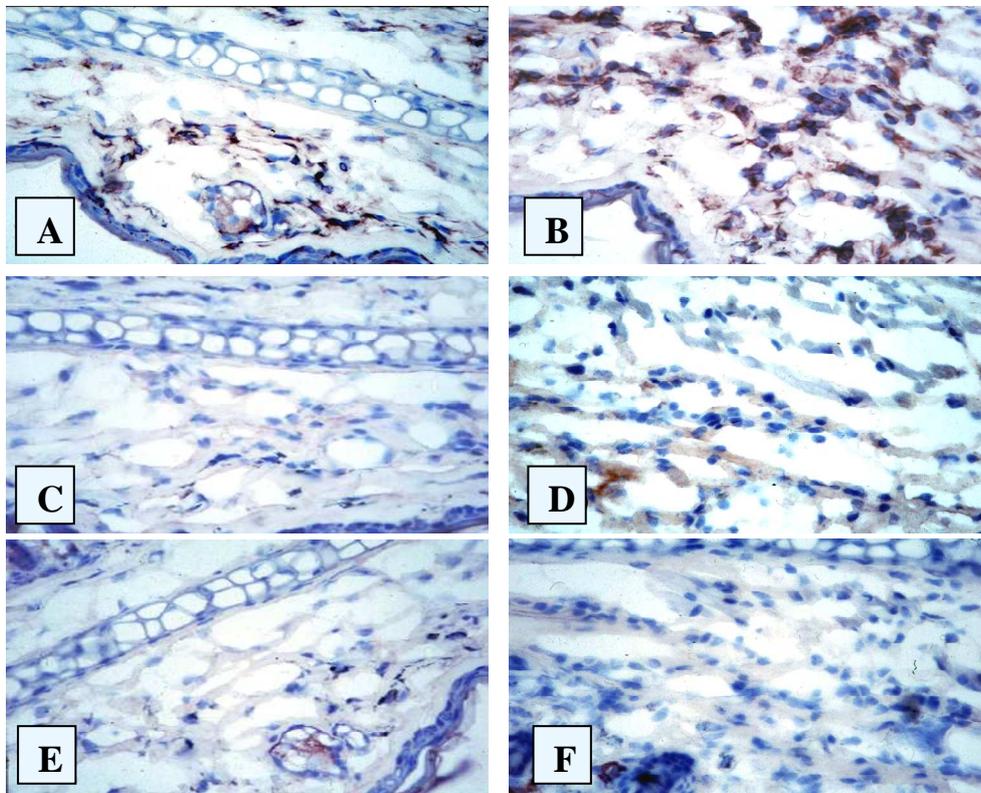


Figure 5. Immunohistological detection of CD45⁺ leukocytes and CD3⁺ T cells in ear samples. After DNFB challenge, a strong CD45⁺ leukocyte infiltration was found in HDC^{-/-} mice (B) (n=6), compared to wild type mice (A) (n=6). The DNFB-painted ears of neither HDC^{-/-} (D), nor wild type mice (C) showed elevated CD3⁺ T cell numbers. Normal rat serum was used as negative control for staining the DNFB treated ears of HDC^{-/-} (F) and wild type mice (E).

IL-2, IFN- γ , TNF- α and IL-4 mRNA expression was examined by Q-RT-PCR

Quantitative relationship between the level of gene expression and relative fluorescence data was demonstrated for each examined cytokine genes. Dilution series of a cDNA was used as template and standard curves were generated where the relative fluorescence data was shown as a function of rate of dilution. The correlation coefficient was >0.9 in each of the examined genes (data not shown), suggesting that the reaction conditions applied resulted in comparable real-time RT-PCR data. Standard curves showed linearity, indicating a quantitative relationship between the relative gene expression and relative fluorescence data (data not shown). The expression of IL-2, IFN- γ , TNF- α and IL-4 genes was examined by optimized real-time RT-PCR reactions in the ear samples obtained at 24 and 48 hours after challenge.

In wild type mice (n=4), IL-2 mRNA was undetectable, in contrast with this HDC-/- mice (n=4) constitutively expressed a detectable level of IL-2 mRNA. In the HDC-/- mice, DNFB treatment (n=6) caused a more than 8-fold increase in the level of IL-2 mRNA 24 hours after challenge, however, the quantity of IL-2 mRNA decreased 48 hours after challenge. In contrast with this, in wild type mice (n=6), IL-2 mRNA was not detected 24 hours after challenge and it reached a detectable level only 48 hours after challenge (Figure 6A).

The IFN- γ mRNA level showed a significantly higher increase in HDC-/- mice than in wild type mice 24 hours after challenge. 48 hours after challenge, the IFN- γ mRNA level decreased in HDC-/- mice, while increased in wild type mice (Figure 6B).

The HDC-/- mice constitutively expressed a detectable level of TNF- α , while in wild type mice TNF- α was undetectable. The increase in TNF- α expression was 7-fold in HDC-/- mice 24 hours after the DNFB treatment, and approximately 3.5-fold higher 48 hours after challenge. In the wild type mice, TNF- α mRNA was not detected 24 hours after the treatment, and showed an increase 48 hours after challenge (Figure 6C).

The expression of IL-4 mRNA reached a detectable level in HDC-/- mice but not in wild type mice. The DNFB treatment of HDC-/- mice produced a moderate increase of IL-4 mRNA expression 24 hours after challenge, and the increase in IL-4 mRNA expression was 5-fold 48 hours after the treatment compared to the 24-hour data. In the wild type mice, IL-4 mRNA was not demonstrated 24 hours after challenge, but a detectable amount of mRNA appeared 48 hours after the DNFB treatment (Figure 7).

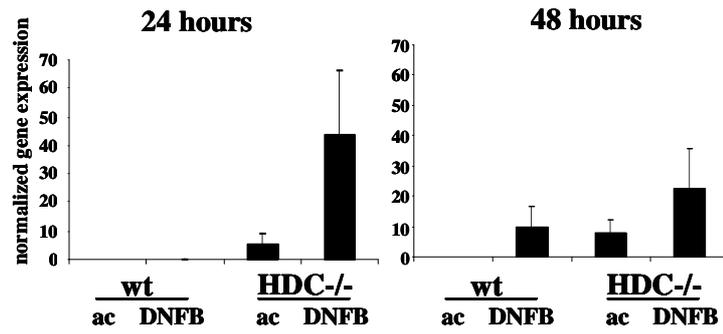
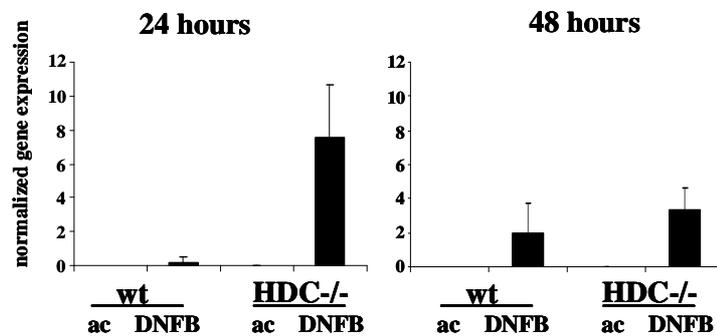
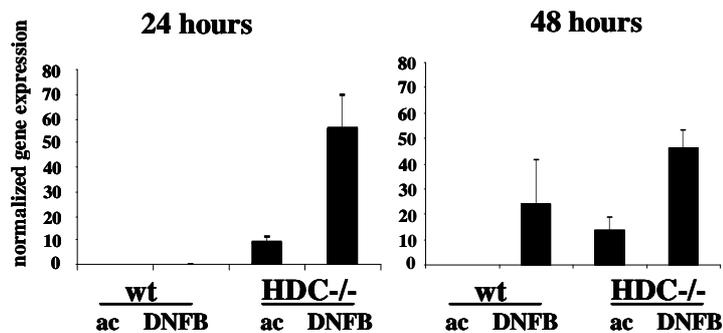
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Figure 6. Real-time RT-PCR analysis of IL-2, IFN- γ and TNF- α mRNA expression in ear samples. HDC-/- mice (n=4) constitutively express higher levels of IL-2 and TNF- α cytokine mRNAs than wild type mice (n=4). The mRNA of these cytokines was not detectable in wild type mice before challenge and 24 hours after challenge. The DNFB treatment caused an increase in IL-2 (A), IFN- γ (B) and TNF- α (C) mRNA expression in HDC-/- mice 24 hours after challenge (n=6). In contrast, 48 hours after challenge, the IL-2, IFN- γ and TNF- α mRNA levels decreased in HDC-/- mice and showed an increase in wild type mice (n=6).

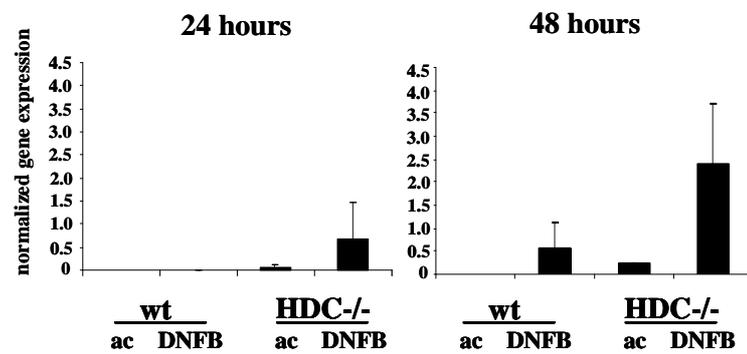


Figure 7. Real-time RT-PCR analysis of IL-4 mRNA expression in ear samples. DNFB treatment of HDC^{-/-} mice produced a moderate increase of IL-4 mRNA expression 24 hours after challenge and a strong increase 48 hours after challenge (n=6). In the wild type mice (n=6), IL-4 mRNA was not detected 24 hours after challenge, but it was demonstrated 48 hours after challenge.

3.4. DISCUSSION

Histamine is an early mediator in inflammatory reactions. It regulates immune responses by enhancing Th2 (IL-4, IL-10) and by inhibiting Th1 (IL-2, IFN- γ , TNF- α) cytokine production (13, 17, 44). In the present study, we investigated whether histamine has a regulatory role in DNFB-induced CHS and whether the lack of histamine modifies the cytokine profile. For that we used the model of HDC^{-/-} mice.

We found in histamine deficient mice, that DNFB induced a more intense CHS reaction than in wild type mice. The DNFB-induced increase of the ear thickness was significantly higher in the HDC^{-/-} mice 24 hours after challenge than in wild type mice. Forty-eight hours after challenge, the ear thickness was still higher in HDC^{-/-} mice, but the difference was not significant between the two groups. After the DNFB challenge the percentages of CD3⁺, CD4⁺ and CD8⁺ T cells in the DLN of sensitization area were significantly lower, those of CD45R⁺ B cells were significantly higher in HDC^{-/-} mice than in wild type mice. Similar differences were found in the DLNs of the untreated HDC^{-/-} and wild type mice. Consequently, these differences do not seem to be due to the DNFB treatment, but they are rather associated with the lack of histamine in HDC^{-/-} mice.

The inflammatory reaction in the ear skin of the mice was also studied. We found that 24 hours after challenge the number of infiltrating cells and the degree of edema was higher in the HDC^{-/-} than in the wild type mice. In hapten challenge sites, neutrophils recruit CD8⁺ T cells that subsequently produce cytokines mediating the hypersensitivity response (32, 45). Using HDC^{-/-} mice Hirasawa *et al* found that histamine plays a negative regulatory role for the neutrophil infiltration *via* H2R receptor in allergic inflammation (18). It has been reported that in the skin of HDC^{-/-} mice the expression of H1R and H2R receptors is very sensitive to histamine levels and both receptors are downregulated in the skin of HDC^{-/-} mice (46). These results suggest that histamine might inhibit neutrophil infiltration in wild-type mice *via* H2R receptors and the lack of histamine favors a strong granulocyte and macrophage infiltration in HDC^{-/-} mice.

We observed an increase of the ear thickness and relatively few infiltrating cells in wild type mice 24 hours after the challenge. In the early phase of elicitation of CHS (3-24 hours after challenge), release of serotonin and TNF- α from mast cells and platelets results in an increased vascular permeability and tissue swelling (26-28). These data indicate that the increase of the ear thickness is mainly due to edema formations that occur 24 hours after challenge.

We also observed that HDC^{-/-} mice constitutively express higher levels of IL-2, TNF- α and IL-4 mRNAs than wild type mice. These findings suggest that endogenous histamine downregulates the production of IL-2, TNF- α and IL-4. It is known that CD8⁺ Tc1 cells mainly produce Th1 type cytokines. In our study, DNFB treatment caused higher levels of Th1 cytokines (IL-2, IFN- γ , TNF- α) in HDC^{-/-} mice 24 and 48 hours after challenge, and a higher level of Th2 cell cytokine (IL-4) mRNA 48 hours after challenge compared to wild type mice.

Challenge with antigen in sensitized mice induces local recruitment of T cells. These antigen-specific T cells produce inflammatory cytokines, which induce ear swelling and other inflammatory processes in the later phase of elicitation (48-72 hours after challenge). We observed a very early Th1 cytokine response in HDC^{-/-} mice, followed by the increased levels of IL-2, IFN- γ and TNF- α mRNAs 24 hours after DNFB challenge. In these mice, the high levels of Th1 cytokines might contribute to the very early increase of the ear thickness and the inflammatory response demonstrated by immunohistology.

We also showed that in the early phase of elicitation, the ear thickness was greater in HDC^{-/-} mice than in wild type mice. Twenty-four hours after challenge, the levels of Th1 cytokine mRNAs were significantly higher in the ear samples of histamine deficient mice compared to wild type mice. These data suggest that histamine might have a suppressive effect on the production of Th1 cytokines and, consequently, on the limitation of the inflammatory response. In the later phase of elicitation, there was no significant difference in the ear swelling in the two groups. Forty-eight hours after challenge a significant increase of Th1 cytokine mRNAs was observed in wild type mice, which was comparable with that seen in HDC^{-/-} mice at 24 hours after challenge. The levels of Th1 cytokine mRNAs in HDC^{-/-} mice 48 hours after challenge were higher than those observed in wild type mice, however, in the HDC^{-/-} mice significantly increased IL-4 levels were also demonstrated. Recent studies have shown that both Th1 and Th2 T cells are involved in the regulation of contact hypersensitivity. IL-4 is a Th2 cytokine that plays an important role during the elicitation phase of CHS, and has a role in mediation of the inflammation (36).

Ohtsu *et al* has reported that in the CHS response the ear thickness of HDC^{-/-} mice was not significantly different from that of wild type mice (41). However, in their experiments another sensitizing agent, trinitrochlorobenzene was used in very high concentrations. We showed, using flow cytometry, immunohistology, and real-time RT-PCR, that DNFB induced a more intense inflammation in HDC^{-/-} mice than in wild type mice. The

discrepancy between their and our results might be explained by the different experimental conditions.

Our data suggest that histamine has an important role both in the early and in the later phase of CHS reaction. The lack of histamine seems to be responsible for a very intense Th1 type response in the early phase and also for a strong Th2 response in the late phase of CHS.

Histamine is known to inhibit Th1 lymphocyte functions such as production of IL-2, IFN- γ *via* H2R receptors, and to enhance Th1-type responses by triggering the H1R receptors (20,47) Fitzsimons *et al* demonstrated that in the skin of HDC^{-/-} mice the H1R and H2R receptors are downregulated which might be due to the prolonged histamine deficiency (46). We found a very early and high Th1 cytokine response after antigen challenge that might be caused by histamine deficiency. These data indicate that endogenous histamine can downregulate the CHS reaction *via* H2R receptor in wild type mice. The lack of histamine causes a downregulation of the H2R receptors in HDC^{-/-} mice thereby leading to a higher Th1 cytokine response compared to wild type mice. These results suggest that in the histamine deficient mice, the Th1/Th2 balance is modulated towards Th1 dominance.

In our study, we demonstrated that histamine is involved in the regulation of delayed type hypersensitivity. Using histamine deficient mice we showed, that histamine plays a suppressive immunoregulatory role in the DNFB induced CHS response.

4. INTRANASAL PHOTOTHERAPY IN SEASONAL ALLERGIC RHINITIS

4.1. INTRODUCTION

Allergic rhinitis is a common inflammatory disease that causes major illness and disability worldwide. The prevalence of AR was found to be around 25% in a study on the general population in Europe (8,9).

We recently showed that intranasal phototherapy is an effective treatment for allergic rhinitis (48). Rhinophototherapy with low doses of mixed ultraviolet and visible light significantly improve the clinical symptoms of AR by acting at multiple points such as induction of T-cell and eosinophil apoptosis and suppression of release of mediators like eosinophil cationic protein and interleukin 5.

Guidelines issued by the Allergic Rhinitis and its Impact on Asthma (ARIA) group recommend the use of second generation antihistamines as first-line treatment for AR (10,49). The newer-generation oral antihistamines such as desloratadine, fexofenadine and levocetirizine have demonstrated efficacy in reducing the symptoms of AR, including rhinorrhea, nasal itching and sneezing, and in some clinical studies nasal congestion (50,51). Fexofenadine is a non-sedating antihistamine, has a rapid onset and a long duration of action (52). In addition to blocking H1 receptors, it has been shown to reduce allergic inflammatory responses mediated by mast cells, basophils, epithelial cells, eosinophils and lymphocytes (53).

The use of second-generation antihistamines in the treatment of seasonal allergic rhinitis (SAR) is well established (53,54). However, in clinical practice, SAR symptoms are not always satisfactory controlled by medication and some patients fail to respond to treatment (55). A new phototherapeutic device has been developed at the University of Szeged, emitting a combination of low dose UVB, UVA and visible light for the treatment of allergic rhinitis (56). The aim of this pilot study was to compare the efficacy of intranasal phototherapy with that of the new generation antihistamine, fexofenadine HCl in seasonal allergic rhinitis.

4.2. MATERIALS AND METHODS

Patients and study design

A randomized open study was conducted in patients with a history of at least 2 years of moderate-to-severe ragweed-induced allergic rhinitis. Positive skin prick test results and an elevated level of ragweed-specific IgE antibody confirmed the diagnosis. The Ethical Committee of University of Szeged approved the protocol. All patients gave their written informed consent. We excluded potential subjects from the study if they had any significant nasal structural abnormalities, had asthma, or upper or lower respiratory infection within 4 weeks before the beginning of the study or had used any of the following drugs: intranasal corticosteroids within 2 weeks, systemic corticosteroids within 4 weeks, membrane stabilizers within 2 weeks, antihistamines within 1 week, nasal decongestants within 3 days or immunotherapy within 5 years before beginning of the study.

The patients were enrolled after the beginning of the ragweed season, when the pollen counts were higher than $50/\text{m}^3$ in the Szeged area. Thirty-one patients with moderate-to-severe symptoms were randomly assigned to receive either intranasal phototherapy (5% UVB, 25% UVA and 70% visible light) 3 times a week for 2 weeks ($n=18$), or 180 mg fexofenadine HCl per day for 2 weeks ($n=13$), with a randomization ratio of 3 to 2. Each intranasal cavity was treated with gradually increasing doses (starting dose: $1.08 \text{ J}/\text{cm}^2$, maximal dose: $1.62 \text{ J}/\text{cm}^2$), the irradiations were performed with the Rhinolight 180 mW lamp (Rhinolight Ltd, Szeged, Hungary). The dose was raised by $0.27 \text{ J}/\text{cm}^2$ at every second treatment.

Each patient kept a daily diary of symptoms on a scale of 0 to 3 (0 indicating no symptoms and 1, 2, 3 indicating mild, moderate and severe symptoms, respectively) for nasal obstruction, nasal itching, rhinorrhea, sneezing and palate itching during the treatment. Total nasal score (TNS), a sum of scores for nasal symptoms (nasal obstruction, itching, rhinorrhea and sneezing) was also calculated.

Statistical analysis

Repeated measures ANOVA test was used to assess the statistical significance of clinical symptom changes and the overall efficacy. The *post hoc* analysis (Dunnett test) revealed the differences between the time points in each treatment group. The percentage changes from baseline in TNS were compared using Fisher exact two tailed test. Value of $P<0.05$ was considered statistically significant.

4.3. RESULTS

Eighteen patients (12 women, 6 men; ages ranged from 18 to 58 years, mean age: 40.67) received intranasal phototherapy and thirteen patients (8 women, 5 men; ages ranged from 18 to 55 years, mean age: 40.00) received 180 mg fexofenadine HCl per day. The 2 groups did not differ significantly in TNS at the beginning of treatment period ($P=0.236$). The baseline TNS (mean \pm SD) was 8.61 \pm 2.64 in the rhinophototherapy group, and 7.46 \pm 2.57 in the fexofenadine HCl group. The mean scores (\pm SD) of each parameter are presented in Table 1.

Parameter	Treatment group	Day 1	Day 7	Day 14	p
Sneezing	Rhinophototherapy	2.278 \pm 0.669	1.889 \pm 0.963	1.000 \pm 1.085	0.0001
	Fexofenadine HCl	2.077 \pm 0.641	1.308 \pm 0.855	1.385 \pm 0.961	0.0173
Rhinorrhea	Rhinophototherapy	2.444 \pm 0.705	2.167 \pm 0.924	1.444 \pm 0.705	0.0001
	Fexofenadine HCl	1.846 \pm 0.801	1.462 \pm 0.660	1.923 \pm 0.954	NS
Nasal itching	Rhinophototherapy	1.833 \pm 1.200	1.167 \pm 1.200	0.667 \pm 0.907	0.0001
	Fexofenadine HCl	1.846 \pm 1.068	1.000 \pm 1.000	1.385 \pm 1.044	NS
Nasal obstruction	Rhinophototherapy	2.056 \pm 0.873	2.056 \pm 0.873	1.389 \pm 0.850	0.0028
	Fexofenadine HCl	1.692 \pm 1.109	1.077 \pm 0.954	1.692 \pm 0.947	NS
Palate itching	Rhinophototherapy	1.667 \pm 1.138	0.778 \pm 0.878	0.444 \pm 0.705	0.0001
	Fexofenadine HCl	1.154 \pm 1.405	0.846 \pm 1.144	1.231 \pm 1.363	NS
TNS	Rhinophototherapy	8.611 \pm 2.638	7.278 \pm 3.006	4.556 \pm 2.854	0.0001
	Fexofenadine HCl	7.462 \pm 2.570	4.846 \pm 2.304	6.385 \pm 3.176	NS

Table 1. Mean scores (\pm SD) of each parameter at the three time points. P values represent the difference between the data at day 14 compared to day 1 in each group (NS: not significant).

The changes from mean baseline values (\pm SE) are shown at day 1, day 7, day 14 in the rhinophototherapy group (Figure 8, right panel). In all of the parameters the scores decreased significantly at the end of treatment compared with day 1 for all of the parameters: sneezing ($P=0.0002$), rhinorrhea ($P=0.0004$), nasal itching ($P=0.0003$), nasal obstruction ($P=0.0014$) and palate itching ($P=0.00002$) (Figure 8 A, B, C, D, E, respectively). In the fexofenadine HCl group (Figure 8, left panel) none of the symptoms improved significantly ($P> 0.05$) at the end of the study except sneezing ($P=0.007$) (Figure 8A). TNS was significantly decreased in the rhinophototherapy group ($P<0.0001$), but no significant difference was observed in the fexofenadine HCl group after 2 weeks of treatment compared to the baseline ($P=0.35$) (Figure 8F).

When we compared the two treatment groups, we did not find significant differences in any of the parameters between the rhinophototherapy group and the fexofenadine HCl group. However, the improvement in the rhinitis symptoms was more pronounced in the rhinophototherapy group compared to the fexofenadine HCl group, but this difference was not statistically significant at the end of the study.

We assessed the changes from baseline in TNS at the end of the study. TNS-25, TNS-50 and TNS-75 correspond to the percentages of responders at day 14 with TNS improvement of more than 25%, 50% and 75%, respectively. If the patient's TNS was reduced by less than 25%, the patient was classified as nonresponder.

After 2 weeks of intranasal phototherapy, there were 15 patients (83.3%) with more than 25% improvement in TNS and 11 patients (61.1%) with more than 50% improvement in TNS compared to the baseline. In contrast to this only 4 patients (30.8%) exhibited more than 25% improvement in TNS and 2 patients (15.4%) showed more than 50% improvement in TNS in the fexofenadine HCl group after last treatment (Figure 9). We found that the ratio of patients with both TNS-25 ($P=0.0075$) and TNS-50 ($P=0.025$) were significantly higher in the rhinophototherapy group compared to the fexofenadine HCl group. There was no significant difference in TNS-75 between the two groups (Figure 9).

Intranasal phototherapy was overall well tolerated. The only side effect was dryness of the nasal mucosa, which occurred in all patients in the rhinophototherapy group and in two patients in the fexofenadine HCl group. All patients scored the dryness as mild except one in the rhinophototherapy group, and were controlled by emollients. In the case of this patient in the rhinophototherapy group one treatment was skipped. All patients completed the study.

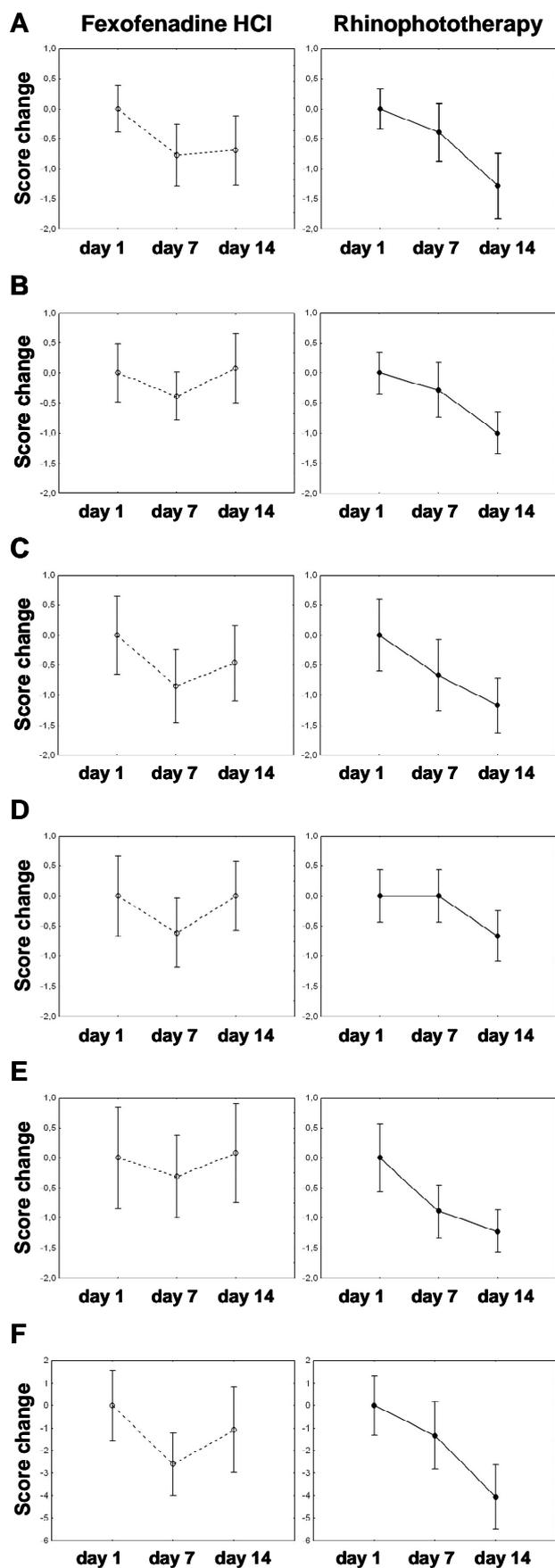


Figure 8. Changes from mean baseline values (\pm SE) for sneezing (A), rhinorrhea (B), nasal itching (C), nasal obstruction (D), palate itching (E) and TNS (F) in the rhinophototherapy group (right panel) and in the fexofenadine HCl group (left panel).

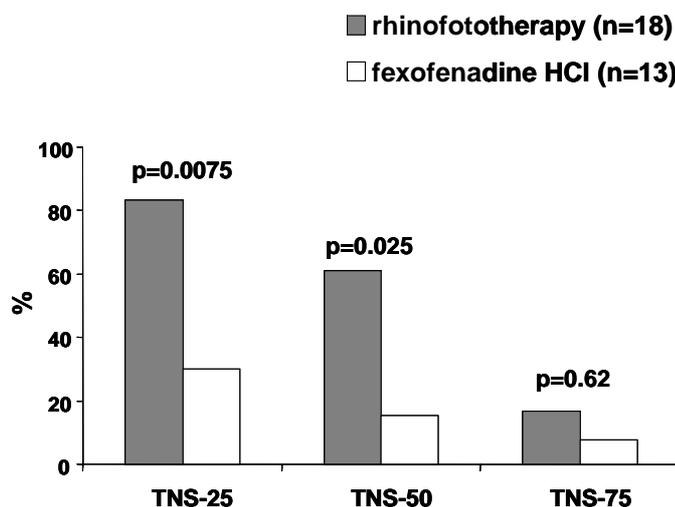


Figure 9. Percentages of responders with TNS improvement of more than 25%, 50% and 75% at the end of the study.

4.4. DISCUSSION

In this pilot study, we found that intranasal phototherapy is more effective than fexofenadine HCl in reducing clinical symptoms in patients with moderate-to-severe SAR. In the rhinofototherapy group, all symptoms improved significantly, in contrast to this none of the scores decreased significantly in the fexofenadine HCl group at the end of the 2 weeks of treatment, except sneezing.

Second generation antihistamines are recommended as first-line therapy for seasonal allergic rhinitis (10,49). In randomized studies with great number of patients fexofenadine HCl exhibited significant improvement in SAR (52,57). The low number of patients involved in our study may account for the results obtained for the fexofenadine HCl group. However, the efficacy of rhinofototherapy in reducing majority of symptoms associated with SAR suggests a more powerful treatment effect.

The mechanism of action involved in the therapeutic effect of rhinofototherapy was investigated in previous studies (48,58,59). We have also published that nasal mucosa exposed to UV light possess the capacity to repair DNA damage (60,61). Nasal dryness induced by allergic inflammation occurs in patients with active symptoms of rhinitis. However, higher number of patients with mild dryness of the nasal mucosa was observed after rhinofototherapy compared to the fexofenadine HCl treatment. We are currently investigating the drying effect of UV with different wavelength on the nasal mucosa. Further studies are needed to define the therapeutic potential of intranasal phototherapy and to determine its application as chronic treatment for perennial allergic rhinitis and possibly to other inflammatory diseases.

5. CONCLUSION

Since its discovery at the beginning of the 20th century, histamine has been established to play a key pathophysiological regulatory role in various immunological functions (1). However, the precise role of histamine is still uncertain. In the last couple of years the role of endogenous histamine has been extensively studied in allergy, asthma, and various autoimmune diseases using histamine deficient mice.

Histidine decarboxylase deficient (HDC^{-/-}) mice were developed about a decade ago by Ohtsu *et al* (3). In these mice the levels of histamine in various tissues are much lower than those in wild type mice. We at first expected that the contact hypersensitivity response would be suppressed in HDC^{-/-} mice. Surprisingly we found that the DNFB induced CHS is more intense in histamine deficient mice than in wild type mice. We provided here the first evidence that histamine can regulate negatively the immunologic response in contact dermatitis. In accordance with our results, the regulatory functions of endogenous histamine have been recently reported by other research groups using experimental animal models with various allergic and autoimmune diseases.

In a different experimental model, chronic allergic contact dermatitis was induced by repeated challenge of diphenylcyclopropenone (DCP) on the back of mice. Seike *et al* found that daily epicutaneous application of DCP induced more intense eczematous lesions of wild-type mice compared to HDC^{-/-} mice (62, 63). Their results suggest that histamine facilitate the development of chronic allergic contact dermatitis induced by repeated challenge with the contact allergen.

Allergic asthma is a complex disease associated with airway hyper-responsiveness (AHR) and chronic airway inflammation. Koarai *et al* examined the role of endogenous histamine in allergic airway eosinophil recruitment and AHR in HDC^{-/-} mice (64). Interestingly they found that the AHR was not suppressed in the HDC^{-/-}-mice compared to wild type mice, however, the proliferation of eosinophils was significantly reduced in the knockout mice. At the same time Kozma reported that the AHR was significantly attenuated in HDC^{-/-} mice (65). The differences in the results of the two groups were explained by the different strains and protocols that they used.

Goblet cell hyperplasia and mucus overproduction are important features of bronchial asthma. In a recent study of Yamauchi *et al* demonstrated that goblet cell

hyperplasia was enhanced in HDC^{-/-} mice (66). They also found significant increases of alveolar macrophages and lymphocytes in the bronchoalveolar lavage fluid (BALF) in HDC^{-/-} mice, and the concentration of TNF α in BALF was significantly higher compared to wild-type mice (67).

The role of histamine in allergic rhinitis was also studied in HDC^{-/-} mice (68). It was found that the intranasal administration of antigen caused a significant increase of nasal sneezing and nasal rubbing, the symptoms of rhinitis in mice. However, the number of sneezing in wild-type mice was significantly higher than in the knockout mice.

Experimental autoimmune encephalomyelitis (EAE) is a prototypic Th1-mediated disease with similarities to human multiple sclerosis (69). It was shown that EAE is significantly more severe in HDC^{-/-} mice with diffuse inflammatory infiltrates compared to wild-type mice. Endogenous histamine appeared to regulate the autoimmune response in EAE and to limit immune damage to the central nervous system.

In our study we used HDC^{-/-} mice and we demonstrated that histamine plays a negative regulatory role in contact hypersensitivity response.

Allergic rhinitis is the most frequent allergic disease affecting 10-20% of the population worldwide (4). Second generation antihistamines are the first-line treatments in AR, however the treatment of allergic rhinitis is occasionally unsatisfactory and some patients fail to respond to the treatment. Using experimental mouse models, it has been shown that H1R antagonists failed to completely suppress nasal allergic symptoms.

In our present work, we compared the efficacy of a second generation antihistamine with that of a new therapeutic device intranasal phototherapy in seasonal allergic rhinitis. Intranasal phototherapy or rhinophototherapy has been recently developed emitting combined UVA, UVB and visible light at the University of Szeged. Previously, rhinophototherapy has been shown to be effective in controlling rhinitis symptoms in moderate-to-severe SAR. The effect of phototherapy has been recently evaluated for the treatment of nasal polyposis, a chronic inflammatory disease of the upper airways (70). Bella *et al* reported that narrow-band UVB treatment represent a potential new option for the management of nasal polyps.

Here we showed that intranasal phototherapy may be an alternative treatment for patients with allergic rhinitis not controlled by antihistamines.

In conclusion, the pathophysiological role of histamine in immunoregulation is a much more complex story than expected. New evidences about the diverse functions of endogenous histamine and its receptors can offer an optimistic perspective for novel therapeutics.

6. SUMMARY

6.1. HISTAMINE IN CONTACT HYPERSENSITIVITY

In the present study, we investigated whether histamine has a regulatory role in DNFB induced CHS and whether the lack of histamine modifies the cytokine profile. In HDC^{-/-} histamine deficient mice we found, that DNFB-induced CHS is more intense than in wild type mice. The DNFB induced increase of the ear thickness was significantly higher in the HDC^{-/-} mice 24 hours after challenge than in wild type mice. Forty-eight hours after challenge, the ear thickness was still higher in HDC^{-/-} mice, but the difference was not significant between the two groups.

We also found that 24 hours after challenge, the number of infiltrating cells and the degree of edema in the ear skin was higher in the HDC^{-/-} mice than in the wild type mice.

Furthermore we showed that HDC^{-/-} mice constitutively express higher levels of IL-2, TNF- α and IL-4 mRNAs than wild type mice. We observed a very early Th1 cytokine response in HDC^{-/-} mice, followed by the increased levels of IL-2, IFN- γ and TNF- α mRNAs 24 hours after DNFB challenge.

We provided here the first evidence that histamine negatively regulates the immune responses in contact dermatitis. In accordance with our results, the negative regulatory functions of endogenous histamine were reported by other research groups using experimental animal models with various allergic and autoimmune diseases. New evidences about the diverse functions of endogenous histamine and its receptors can offer an optimistic perspective for novel therapeutics.

6.2. INTRANASAL PHOTOTHERAPY IN SEASONAL ALLERGIC RHINITIS

In our pilot study, we found that intranasal phototherapy (rhinophototherapy) is more effective than fexofenadine HCl in reducing clinical symptoms in patients with moderate-to-severe seasonal allergic rhinitis. Further large scale studies are needed to define the therapeutic potential of rhinophototherapy and to determine its application as chronic treatment for perennial allergic rhinitis.

7. REFERENCES

1. Zampeli E, Tiligada E. The role of histamine H4 receptor in immune and inflammatory disorders. *Br J Pharmacol* **2009**; 157: 24-33.
2. Smuda C, Bryce PJ. New Developments in the Use of Histamine and Histamine Receptors. *Curr Allergy Asthma Rep* **2011**; 11: 94-100.
3. Ohtsu H, Tanaka S, Terui T et al. Mice lacking histidine decarboxylase exhibit abnormal mast cells. *FEBS Lett* **2001**; 502: 53-56.
4. Compalati E, Penagos M, Henley K, Canonica GW. Allergy Prevalence Survey by the World Allergy Organization. *J World Allergy Org* **2007**; 19: 82-90.
5. Vocanson M, Hennino A, Rozieres A, Poyet G, Nicolas JF. Effector and regulatory mechanisms in allergic contact dermatitis. *Allergy* **2009**; 64: 1699-1714.
6. Enk AH. Allergic contact dermatitis: understanding the immune response and potential for targeted therapy using cytokines. *Mol Med Today* **1997**; 3: 423-428.
7. Lachapelle J-M, Maibach HI. Patch Testing and Prick Testing. A Practical Guide. Official Publication of the ICDRG. Berlin, Heidelberg: Springer Verlag, 2009.
8. Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. *Eur Respir J* **2004**; 24: 758-764.
9. Bauchau V, Durham SR. Epidemiological characterization of the intermittent and persistent types of allergic rhinitis. *Allergy* **2005**; 60: 350-353.
10. Bousquet J, Khaltaev N, Cruz AA et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* **2008**; 63 Suppl 86: 8-160.
11. Oettgen HC, Geha RS. IgE in asthma and atopy: cellular and molecular connections. *J Clin Invest* **1999**; 104: 829-835.
12. Kayasuga R, Sugimoto Y, Watanabe T, Kamei C. Participation of chemical mediators other than histamine in nasal allergy signs: a study using mice lacking histamine H(1) receptors. *Eur J Pharmacol* **2002**; 449: 287-291.
13. Falus A, Meretey K. Histamine: an early messenger in inflammatory and immune reactions. *Immunol Today* **1992**; 13: 154-156.
14. Kubo Y, Nakano K. Regulation of histamine synthesis in mouse CD4+ and CD8+ T lymphocytes. *Inflamm Res* **1999**; 48: 149-153.
15. Laszlo V, Stohr J, Hegyesi H et al. Increase of histidine decarboxylase in human monocytes during macrophage colony stimulating factor induced in vitro phagocytic differentiation. *Inflamm Res* **1999**; 48 Suppl 1: S85-S86.

16. Szeberenyi JB, Pallinger E, Zsinko M et al. Inhibition of effects of endogenously synthesized histamine disturbs in vitro human dendritic cell differentiation. *Immunol Lett* **2001**; 76: 175-182.
17. Igaz P, Novak I, Lazaar E, Horvath B, Heninger E, Falus A. Bidirectional communication between histamine and cytokines. *Inflamm Res* **2001**; 50: 123-128.
18. Hirasawa N, Ohtsu H, Watanabe T, Ohuchi K. Enhancement of neutrophil infiltration in histidine decarboxylase-deficient mice. *Immunology* **2002**; 107: 217-221.
19. Novak I, Falus A. Molecular biology and role of histamine in physiological and pathological reactions. A review. *Acta Derm Venereol* **1997**; 48: 385-394.
20. Jutel M, Watanabe T, Klunker S et al. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. *Nature* **2001**; 413: 420-425.
21. Askenase PW. Effector and regulatory molecules and mechanisms in delayed type hypersensitivity. In: Middleton E, Ellis EF, Yunginger JW, Reed ChE, Adkinson NF, Busse WW, eds. Allergy. Principles & Practice. St.Louis, Baltimore, Boston, Carlsbad, Chicago, Minneapolis, New York, Philadelphia, Portland, London, Milan, Sydney, Tokyo, Toronto: Mosby, 1998: 323-41.
22. Gerberick GF, Cruse LW, Miller CM, Ridder GM. Selective modulation of B-cell activation markers CD86 and I-Ak on murine draining lymph node cells following allergen or irritant treatment. *Toxicol Appl Pharmacol* **1999**; 159: 142-151.
23. Fujisawa H, Kondo S, Wang B, Shivji GM, Sauder DN. The role of CD4 molecules in the induction phase of contact hypersensitivity cytokine profiles in the skin and lymph nodes. *Immunology* **1996**; 89: 250-255.
24. Gorbachev AV, Fairchild RL. Induction and regulation of T-cell priming for contact hypersensitivity. *Crit Rev Immunol* **2001**; 21: 451-472.
25. Wang B, Feliciani C, Howell BG et al. Contribution of Langerhans cell-derived IL-18 to contact hypersensitivity. *J Immunol* **2002**; 168: 3303-3308.
26. Askenase PW, Bursztajn S, Gershon MD, Gershon RK. T cell-dependent mast cell degranulation and release of serotonin in murine delayed-type hypersensitivity. *J Exp Med* **1980**; 152: 1358-1374.
27. Van Loveren H, Meade R, Askenase PW. An early component of delayed-type hypersensitivity mediated by T cells and mast cells. *J Exp Med* **1983**; 157: 1604-1617.
28. Askenase PW, Geba GP, Levin J et al. A role for platelet release of serotonin in the initiation of contact sensitivity. *Int Arch Allergy Immunol* **1995**; 107: 145-147.
29. Geba GP, Ptak W, Anderson GM et al. Delayed-type hypersensitivity in mast cell-deficient mice: dependence on platelets for expression of contact sensitivity. *J Immunol* **1996**; 157: 557-565.

30. Askenase PW, Tsuji RF. B-1 B cell IgM antibody initiates T cell elicitation of contact sensitivity. *Curr Top Microbiol Immunol* **2000**; 252:171-7.: 171-177.
31. Akiba H, Kehren J, Ducluzeau MT et al. Skin inflammation during contact hypersensitivity is mediated by early recruitment of CD8+ T cytotoxic 1 cells inducing keratinocyte apoptosis. *J Immunol* **2002**; 168: 3079-3087.
32. Martin A, Gallino N, Gagliardi J et al. Early inflammatory markers in elicitation of allergic contact dermatitis. *BMC Dermatol* **2002**; 2: 9.
33. Kondo S, Sauder DN. Epidermal cytokines in allergic contact dermatitis. *J Am Acad Dermatol* **1995**; 33: 786-800.
34. Xu H, DiIulio NA, Fairchild RL. T cell populations primed by hapten sensitization in contact sensitivity are distinguished by polarized patterns of cytokine production: interferon gamma-producing (Tc1) effector CD8+ T cells and interleukin (Il) 4/Il-10-producing (Th2) negative regulatory CD4+ T cells. *J Exp Med* **1996**; 183: 1001-1012.
35. Ulrich P, Grenet O, Bluemel J et al. Cytokine expression profiles during murine contact allergy: T helper 2 cytokines are expressed irrespective of the type of contact allergen. *Arch Toxicol* **2001**; 75: 470-479.
36. Watanabe H, Unger M, Tuvel B, Wang B, Sauder DN. Contact hypersensitivity: the mechanism of immune responses and T cell balance. *J Interferon Cytokine Res* **2002**; 22: 407-412.
37. Belsito DV, Kerdel FA, Potozkin J, Soter NA. Cimetidine-induced augmentation of allergic contact hypersensitivity reactions in mice. *J Invest Dermatol* **1990**; 94: 441-445.
38. Griswold DE, Alessi S, Badger AM, Poste G, Hanna N. Differential sensitivity of T suppressor cell expression to inhibition by histamine type 2 receptor antagonists. *J Immunol* **1986**; 137: 1811-1815.
39. Grob JJ, Castelain M, Richard MA et al. Antiinflammatory properties of cetirizine in a human contact dermatitis model. Clinical evaluation of patch tests is not hampered by antihistamines. *Acta Derm Venereol* **1998**; 78: 194-197.
40. Wiener Z, Andrasfalvy M, Pallinger E et al. Bone marrow-derived mast cell differentiation is strongly reduced in histidine decarboxylase knockout, histamine-free mice. *Int Immunol* **2002**; 14: 381-387.
41. Ohtsu H, Kuramasu A, Tanaka S et al. Plasma extravasation induced by dietary supplemented histamine in histamine-free mice. *Eur J Immunol* **2002**; 32: 1698-1708.
42. Par G, Szekeres-Bartho J, Buzas E, Pap E, Falus A. Impaired reproduction of histamine deficient (histidine-decarboxylase knockout) mice is caused predominantly by a decreased male mating behavior. *Am J Reprod Immunol* **2003**; 50: 152-158.
43. Janossy T, Baranyi L, Knulst AC, Vizler C, Benner R, Vegh P. MHC-specific graft-protective and delayed-type hypersensitivity (DTH) suppressive activity of a CD4+ CD8+, alpha beta T cell receptor (TCR) positive lymphoma isolated from a tolerant mouse. *Immunobiology* **1993**; 188: 172-193.

44. Horvath BV, Szalai C, Mandi Y et al. Histamine and histamine-receptor antagonists modify gene expression and biosynthesis of interferon gamma in peripheral human blood mononuclear cells and in CD19-depleted cell subsets. *Immunol Lett* **1999**; 70: 95-99.
45. Zhang L, Tinkle SS. Chemical activation of innate and specific immunity in contact dermatitis. *J Invest Dermatol* **2000**; 115: 168-176.
46. Fitzsimons CP, Lazar-Molnar E, Tomoskozi Z, Buzas E, Rivera ES, Falus A. Histamine deficiency induces tissue-specific down-regulation of histamine H2 receptor expression in histidine decarboxylase knockout mice. *FEBS Lett* **2001**; 8: 245-248.
47. Ohuchi Y, Ohtsu H, Sakurai E et al. Induction of histidine decarboxylase in type 2 T helper lymphocytes treated with anti-CD3 antibody. *Inflamm Res* **1998**; 47 Suppl 1: S48-S49.
48. Koreck AI, Csoma Z, Bodai L et al. Rhinophototherapy: a new therapeutic tool for the management of allergic rhinitis. *J Allergy Clin Immunol* **2005**; 115: 541-547.
49. Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* **2001**; 108: S147-S334.
50. Morgan MM, Khan DA, Nathan RA. Treatment for allergic rhinitis and chronic idiopathic urticaria: focus on oral antihistamines. *Ann Pharmacother* **2005**; 39: 2056-2064.
51. Bachert C. A review of the efficacy of desloratadine, fexofenadine, and levocetirizine in the treatment of nasal congestion in patients with allergic rhinitis. *Clin Ther* **2009**; 31: 921-944.
52. Hampel F, Ratner P, Mansfield L, Meeves S, Liao Y, Georges G. Fexofenadine hydrochloride, 180 mg, exhibits equivalent efficacy to cetirizine, 10 mg, with less drowsiness in patients with moderate-to-severe seasonal allergic rhinitis. *Ann Allergy Asthma Immunol* **2003**; 91: 354-361.
53. Lehman JM, Blaiss MS. Selecting the optimal oral antihistamine for patients with allergic rhinitis. *Drugs* **2006**; 66: 2309-2319.
54. Plaut M, Valentine MD. Clinical practice. Allergic rhinitis. *N Engl J Med* **2005**; 353: 1934-1944.
55. Nathan RA. The burden of allergic rhinitis. *Allergy Asthma Proc* **2007**; 28: 3-9.
56. Kemeny L, Koreck A. Ultraviolet light phototherapy for allergic rhinitis. *J Photochem Photobiol B* **2007**; 87: 58-65.
57. Prenner BM, Capano D, Harris AG. Efficacy and tolerability of loratadine versus fexofenadine in the treatment of seasonal allergic rhinitis: a double-blind comparison with crossover treatment of nonresponders. *Clin Ther* **2000**; 22: 760-769.

58. Koreck A, Csoma Z, Boros-Gyevi M et al. Inhibition of immediate type hypersensitivity reaction by combined irradiation with ultraviolet and visible light. *J Photochem Photobiol B* **2004**; 77: 93-96.
59. Brehmer D, Schon MP. Endonasal phototherapy significantly alleviates symptoms of allergic rhinitis, but has a limited impact on the nasal mucosal immune cells. *Eur Arch Otorhinolaryngol* **2011**; 268: 393-399.
60. Koreck A, Szechenyi A, Morocz M et al. Effects of intranasal phototherapy on nasal mucosa in patients with allergic rhinitis. *J Photochem Photobiol B* **2007**; 89: 163-169.
61. Mitchell D, Paniker L, Sanchez G et al. Molecular response of nasal mucosa to therapeutic exposure to broad-band ultraviolet radiation. *J Cell Mol Med* **2010**; 14: 313-322.
62. Seike M, Takata T, Ikeda M, Kodama H, Terui T, Ohtsu H. Histamine helps development of eczematous lesions in experimental contact dermatitis in mice. *Arch Dermatol Res* **2005**; 297: 68-74.
63. Seike M, Furuya K, Omura M, Hamada-Watanabe K, Matsushita A, Ohtsu H. Histamine H(4) receptor antagonist ameliorates chronic allergic contact dermatitis induced by repeated challenge. *Allergy* **2010**; 65: 319-326.
64. Koarai A, Ichinose M, Ishigaki-Suzuki S et al. Disruption of L-histidine decarboxylase reduces airway eosinophilia but not hyperresponsiveness. *Am J Respir Crit Care Med* **2003**; 167: 758-763.
65. Kozma GT, Losonczy G, Keszei M et al. Histamine deficiency in gene-targeted mice strongly reduces antigen-induced airway hyper-responsiveness, eosinophilia and allergen-specific IgE. *Int Immunol* **2003**; 15: 963-973.
66. Yamauchi K, Piao HM, Nakadate T et al. Progress in allergy signal research on mast cells: the role of histamine in goblet cell hyperplasia in allergic airway inflammation - a study using the Hdc knockout mouse. *J Pharmacol Sci* **2008**; 106: 354-360.
67. Yamauchi K, Piao HM, Nakadate T et al. Enhanced goblet cell hyperplasia in HDC knockout mice with allergic airway inflammation. *Allergol Int* **2009**; 58: 125-134.
68. Rahman MA, Inoue T, Ishikawa T, Yatsuzuka R, Ohtsu H, Kamei C. Involvement of chemical mediators in nasal allergic responses of HDC-KO mice. *Eur J Pharmacol* **2007**; 567: 245-251.
69. Musio S, Gallo B, Scabeni S et al. A key regulatory role for histamine in experimental autoimmune encephalomyelitis: disease exacerbation in histidine decarboxylase-deficient mice. *J Immunol* **2006**; 176: 17-26.
70. Bella Z, Kadocsa E, Kemeny L, Koreck A. Narrow-band UVB phototherapy of nasal polyps: results of a pilot study. *J Photochem Photobiol B* **2010**; 100: 123-127.

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