THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

Histamine regulates multiple functional activities of murine and monocyte-derived human dendritic cells via different receptors

by
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CONTENTS

1. ABBREVIATIONS

5/4E8	mouse T cell hybridoma cell line from Balb/c mouse strain
4-MH	4-methylhistamine
ABP1	amiloride binding protein 1
ANOVA	Analysis of Variance
APC	Allophycocyanin
Balb/c	inbred mouse strain
BP	band pass
BSA	bovine serum albumin
cAMP	cyclic adenosine 3’5’-monophosphate
CCL_	chemokine (CC motif) ligand
CCR_	chemokine (CC motif) receptor
CD	Cluster of Differentiation
cDNA	complementer DNA
cDC	conventional dendritic cell
CFA	Complete Freund’s Adjuvant
CI	Cell index value
C_T	Cycle threshold
Da	dalton
DAO	diamine-oxidase
DM	dicroic mirror
DMEM	Dulbecco’s Modified Eagle’s Medium
DMSO	dimethyl-sulfoxide
dNTP	deoxyribonucleotide triphosphate
DC	dendritic cell
DNA	deoxyribonucleic acid
FACS	fluorescence activated cell sorter

FCS	fetal calf serum
FITC	fluorescein isothiocyanate
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
Gata-3	GATA binding protein 3, transcription factor
GM-CSF	Granulocyte-Monocyte Colony Stimulating Factor
H_1R	histamine H1 receptor
H_2R	histamine H2 receptor
H_3R	histamine H3 receptor
H_4R	histamine H4 receptor
HDC	L-histidine decarboxylase
HNMT	histamine N-methyltransferase
HR	histamine receptor
HRP	horseradish peroxidase
IDC	immature dendritic cell
IFNγ	interferon-gamma
IL-	interleukin
iNKT cell	invariant natural killer T cell
JNJ7777120	H_4R antagonist developed by Johnson&Johnson
JNJ10191584	H_4R antagonist developed by Johnson&Johnson
kDa	kilodalton
KO	knockout
LPS	lipopolysaccharide
LC	Langerhans cell
LP	long pass
MACS	magnetic cell separation
MAPK	mitogen-activated protein kinase
MDC	mature dendritic cell

MHC	major histocompatibility complex
mRNA	messenger RNA
NKT	natural killer T cell
OCT3	organic cation transporter 3
OVA	ovalbumin
PAMP	Pathogen-Associated Molecular Pattern
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
pDC	plasmacytoid dendritic cell
PE	Phycoerythrin
PFA	Paraformaldehyde
PMSF	phenylmethanesulfonyl fluoride
PRR	Pattern Recognition Receptor
PVDF	polyvinylidene fluoride
RNA	ribonucleic acid
RPMI	medium developed by Moore and colleagues in Roswell Park Memorial Institute
SD	standard deviation
SDS	Sodium- Dodecil-Sulphate
SEM	Standard Error of Mean
SLC22A3	solute carrier family 22, member 3
SP	short pass
SPF	specific pathogen free
SV129	not inbred mouse strain
T-bet	T-box expressed in T cells, T-box protein 21, transcription factor
Th	T helper cell
TLR	Toll-like Receptor
TNF-α	tumor necrosis factor-alpha

Treg	regulatoric T cell
WT	wild type
Z	impedance

2. INTRODUCTION

Reactions of the immune system occur under in a delicate balance between responding to environmental and pathogenic challenges while maintaining tolerance to self-tissues. Dendritic cells (DCs) form a heterogeneous fraction of rare hematopoietic cells that belong to innate immunity but act in close collaboration with cells of the adaptive immune system. By processing and presenting antigen and migrating from the circulation and tissue sites of antigen uptake to the meeting points of cellular interactions, i.e. peripheral lymph nodes these cells play critical role in the initiation and polarization of immune responses and in the development of effector and memory cells. While continuously patrolling between peripheral tissues and lymphoid organs and sampling their environment numerous metabolites, cytokines, chemokines and other soluble mediators may influence the properties and functions of DCs.

A rapidly growing body of evidence highlighted that histamine, a small biogenic amine, is involved in the regulation of DC differentiation and functions and consequently T cell-mediated inflammation. Initially, this potent bioamine with multiple activities in various pathological and physiological conditions was recognized as an inflammatory mediator released by mast cells, basophils and other cell types, including DCs themselves. Indeed, histamine has been implicated in the regulation of different diseases associated with acute or chronic inflammation, such as allergy, asthma, and its associated diseases, autoimmunity and even tumors.

Improving the quality of life of a patient with any of the above mentioned diseases and the development of novel therapeutic e.g. more specific histamine receptor (HR) ligands, and/or diagnostic strategies requires a better understanding of the crosstalk of cells, which respond to histamine. As DCs act as special sentinels of the immune system linking innate and adaptive immune responses and play a crucial role in regulating inflammatory conditions they provide a promising therapeutic target in disease conditions, where histamine is involved. Thus, the discovery of molecular mechanism relevant to histamine actions on DCs has especial relevance to the development of more effective agents and novel means to modulate inflammation and/or other diseases. Clinical research in this field is still in progress and the already developed HR blocking agents offer a promise of improved treatment outcomes. However, despite intensive research efforts and several clinical trials, none of the new experimental therapeutics and inhibitors has been proved to significantly improve the

conditions of patients suffering from inflammatory diseases. Therefore, novel approaches to decipher histamine biology are still critical.

The purpose of our study was to investigate the effects of histamine on various functional activities of mouse and human DCs. Migration, adhesion, cytokine production and the antigen presenting function of splenic and bone marrow-derived murine DCs was investigated *in vivo* and *in vitro*. Beyond these studies we also investigated the possible impact of the lastly discovered H_4R in mediating histamine effects. Using human monocyte-derived DC subsets we tested how histamine influences the development and functional activities of $CD1a^-$ and $CD1a^+$ DC subsets and identified the role of histamine on the cytokine and chemokine production as well as migration of these cells.

3. THEORETICAL BACKGROUND

The thesis built around two topics. In the first part we investigated the role of a small, pleiotropic biogenic amine, histamine and H_4R in murine dendritic cell functions. Then we studied the effects of histamine on the differentiation and activities of two, developmentally related and *in vivo* relevant human monocyte-derived DC subsets ($CD1a^-$ and $CD1a^+$ cells) generated from $CD14^{high}$ monocytes.

3.1. DCs

The mammalian organism is protected against invading microorganisms at the interfaces of the external environment (skin, respiratory tracts, gastrointestinal mucosa) by special sentinels, called DCs (Figure 1.).

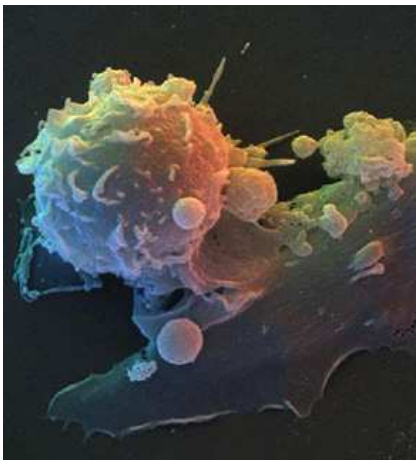


Figure 1.

DCs, sentinels of the immune system
(dendriticcellresearch.com)

Discovery of DCs

This cell type with characteristic shape and projections was first described in 1868 by Peter Langerhans in the skin, however he could not define their origin. In any case, it was certain that their number was significantly lower compared to other blood cells. Based on their morphology and localization the cells were thought to be sensory nerve endings. Almost 100 years after this discovery in 1973 Ralph Steinmann and Zanvil Cohn proved that the skin cells with dendrites belong to the immune system and postulated their role in regulating immunological processes. Early DC research was set back by the low incidence of these cells and consequently, the small yield of isolated cells from various tissues. The discovery of growth and differentiation factors in the development and differentiation of blood cells brought about a major breakthrough in the field and enabled the *in vitro* generation of DCs in large quantities [1].

Subsets, origin and life cycle of DCs

Based on the functional, phenotypic and morphological similarity of DCs to macrophages, their myeloid origin was suggested [2]. In line with this Inaba and co-workers demonstrated that granulocytes, macrophages and DCs were able to differentiate from a common progenitor in the presence of GM-CSF [3]. Another convincing argument was provided by Lanzavecchia and Sallusto showing that human monocytes had the potential to differentiate into DCs *in vitro*, which was later confirmed in mice studies by Randolph [4] [5]. However, *in vivo* studies have shown that DCs can develop from both common lymphoid and myeloid progenitors [6].

DCs were initially classified into two groups, which include the steady state conventional DCs (cDCs) and the non-conventional DCs [7]. cDCs could be further divided into migratory and lymphoid DCs. The first group is comprised of cells derived from a common DC progenitor and reside in peripheral tissues such as the skin, lung, intestinal tract, liver and kidney. These DCs have the unique ability to pick up antigens in the periphery and subsequently migrate to the draining lymph nodes, the primary site of antigen presentation. The lymphoid subsets are localized to lymphoid organs such as the thymus, spleen and lymph nodes. Plasmacytoid DCs (pDCs), being classified as non-conventional DCs, are unique in their ability to produce high amounts of type-I IFN, which distinguishes them from

conventional DCs. Furthermore, monocytes can give rise to DCs under inflammatory as well as steady state conditions in peripheral tissues such as the intestine, lung, skin and kidneys and are also able to acquire antigen and activate T cells in lymph nodes [8].

Development of DCs from bone marrow precursors requires several transcription factors and cytokines to give rise to various DC subsets. The cytokines GM-CSF and IL-4 are commonly used for the *in vitro* generation of DCs from $CD14^+$ monocytes [4] [7]. Beside other molecules, the biogenic amine, histamine was also identified as a regulator of DC differentiation [9].

DCs are generated in the bone marrow and they are seeded through the blood stream into peripheral lymphoid and non-lymphoid tissues, where they easily make contact with foreign particles or invading microbes [7] [10]. When continuously sampling their environment, at an immature stage of development, DCs capture different molecules by their cell surface receptors and internalize them via various mechanisms such as phagocytosis, macropinocytosis or receptor-mediated endocytosis [10] [11] [12]. After processing of the antigenic sample it is fixed on the surface of DCs as peptides that are presented by major histocompatibility complex (MHC) molecules to antigen-specific T cells. Immature DCs are characterized by high capability of antigen capture and processing, but low T cell stimulatory capacity [13]. Antigen uptake and inflammatory mediators promote DC maturation and migration to draining lymph nodes where they efficiently trigger an immune response by naive T cells [10] [11]. DC maturation is a complex and highly regulated process which include phenotypic and functional changes such as losing the ability of antigen capture, acquiring an increased capacity for T cell stimulation and upregulation of costimulatory molecules [10] [11]. Furthermore, maturation is accompanied by the modulation of the expression of some chemokine receptors. While CXCR1, CCR1, CCR5 expression is downregulated, the chemokine receptor CCR7 is induced together with DC maturation enabling the mobilization of DCs from the periphery to the lymph nodes [14] [15]. CCL19 (MIP-3ß) and CCL21, the ligands of CCR7 are produced constitutively in the T cell areas of lymph nodes [16] [17] [18] [19]. CCL21/6Ckine molecule expressed in lymphatic vessels also plays a role in directing these cells [20] [21]. Upon arrival to lymphoid compartments DCs are able to activate different subsets of lymphocytes by polarizing them to Th1, Th2, Th17 or Treg directions. This process is highly dependent on DC-derived signals that involve foreign peptide-MHC complexes expressed on the DC surface, co-stimulatory signals and cytokine/chemokine-mediated instructions [22]. The interaction of the T cell receptor (TCR) with its specific ligand is indispensable as a first signal, whereas the second signals are

mediated by B7-1 or B7-2 molecules (CD80, CD86) by triggering T cells through CD28 receptor and/or additional environmental stimuli. The cytokines produced by DCs and T cells act in both directions and modulate the intensity and quality of the immune response (by the differentiation of Th1, Th2, Th17 and Treg cells) [23] [24] [25]. IL-12 or IFNγ produced by DCs can polarize T cells to Th1 direction, the IL-4 or IL-5 cytokines support Th2 polarization, whereas IL-10 induces immune suppression [26] [27] [28]. The antigen presenting function of DCs is not restricted to the presentation of endogenous peptides by MHCI for $CD8^+$ T cells and exogenous peptides by MHCII molecules for $CD4^+$ T cells, as they also present lipids and glycolipids complexed with CD1 membrane proteins and endo- or exogenous antigens with MHCI molecules to elicit $CD8^+$ T cells, called cross-presentation [10] [29] [30] [31].

Due to their multiple functions the proper instructions of DCs to other immune cells is essential for inducing immunological tolerance in the thymus and the periphery, or provoking an effective immune response [32] [33]. Depending on the type of the invading microorganism and the nature of stress factors or danger signals DCs can direct immune responses to various activities.

CD1a, a human DC marker and lipid presenting molecule

In contrast to highly polymorphic classical MHC molecules, members of the CD1 family of MHC-class-I-like glycoproteins, are phylogenetically conserved proteins, specialized to the presentation of hydrophobic ligands, such as self and foreign lipids and glycolipids. The CD1 proteins share sequence homology and overall domain structure with MHC class I molecules, being comprised of a heavy chain that is non-covalently associated with ß2-microglobulin. To our current knowledge five CD1 isoforms can be distinguished and based on their sequence can be further divided into three subgroups. Group1 is composed of CD1a, CD1b and CD1c molecules, group 2 and group 3 contain only one protein, CD1d and CD1e, respectively. Except from CD1e, all isoforms are cell surface proteins. Interestingly, the expression pattern of human CD1 proteins differs from other species, as in humans all five isoforms are present, while in mice only CD1d is expressed. Group 1 molecules preferentially present lipid antigens to „conventional" αß or innate γð T cells, while group 2 molecules (CD1d) present antigens to invariant NKT cells (iNKT) [34].

CD1a, a member of the group 1 family is expressed mainly on DCs, though it was first identified in thymic cells [35]. Since then, its expression was demonstrated on epithelial cells in breast cancer as well [36]. *In vivo* three different DC fractions can be isolated from human

blood: $CD1a^{+}CD11c^{+}$, $CD1a^{-}CD11c^{-}$ and $CD1a^{-}CD11c^{+}$ populations. The double positive population is the direct precursor of LCs, and can be differentiated to LCs *in vitro*. CD1a expression is similar on resting and activated DCs.

3.2. Histamine and HRs

Discovery, synthesis and degradation of histamine

Histamine has been known for more than hundred years, however its physiological and pathological effects are still not completely known.

This small pleiotropic biogenic amine was first synthesized in 1907 by Windaus and Vogt, and after three years Barger and Dale showed it in ergot extract and proved its constrictory and vasodepressor effects. The name histamine was used first by Führer and Fröhlich. The discovery that simple chemicals, like histamine can regulate complex biological processes brought an enormous scientific improvement in biological researches. During a century numerous effects of histamine have been recognized and at the end of the 1930's the first synthetic HR ligands were produced, followed by several new ones, which were widely used yet in the middle of the century [37] [38] [39].

Histamine is composed of 17 atoms with a molecular mass of 111 Da. It is synthesized by the decarboxylation of the L-histidine amino acid catalyzed by L histidine decarboxylase (HDC) enzyme [40] [41] (Figure 2.). This unique enzyme (only HDC is capable of removing carboxyl group) is present almost in every cell type and tissue.

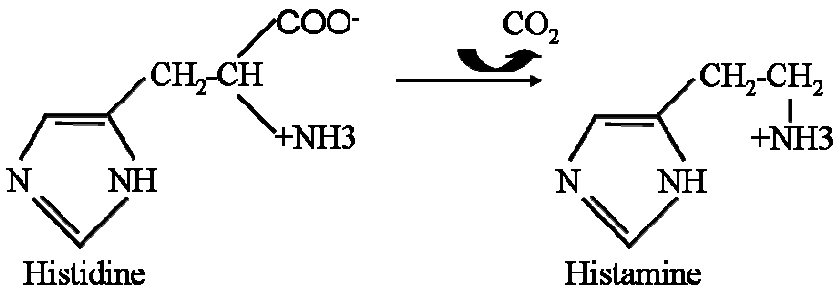


Figure 2.

Biosynthesis of histamine

Histamine is synthesized by the decarboxylation of the histidine amino acid in the process catalyzed by histidine decarboxylase enzyme.

From immunological aspects the most relevant histamine producing cells are mast cells and basophil granulocytes, which not only synthesize this amine *de novo*, but they can store and release it within seconds from their specific intracellular granules after various activation stimuli [37].

Beside the synthesis of histamine its degradation is also important which is performed by two enzymes, the ABP1 (amiloride binding protein 1) also referred to as diamine-oxidase (DAO) and the histamine N-methyltransferase (HNMT) [42]. The extracellularly localized ABP1 creates the inactive imidazole acetaldehyde by removing the terminal amino group, while HNMT convert histamine to N^{τ}-methyl histamine by using S-adenosyl methionine within the cell. Interestingly, tissue expression of the two enzymes is different: ABP1 is found mainly in the intestine, kidney, liver and in the placenta during pregnancy. In contrast, HNMT shows wide tissue distribution (central nervous system, liver, spleen, kidney, intestine, colon, prostate, ovary) [43]. Non-neuronal monoamine transporters, which actively remove monoamines from the extracellular space, have been described in the transportation of histamine. SLC22A3 (solute carrier family 22, member 3) also referred to as OCT3 (organic cation transporter 3), is a common molecule in histamine transport [37].

Tissue concentration of histamine depends on the local amount and activity of these enzymes and transporters (Figure 3.).

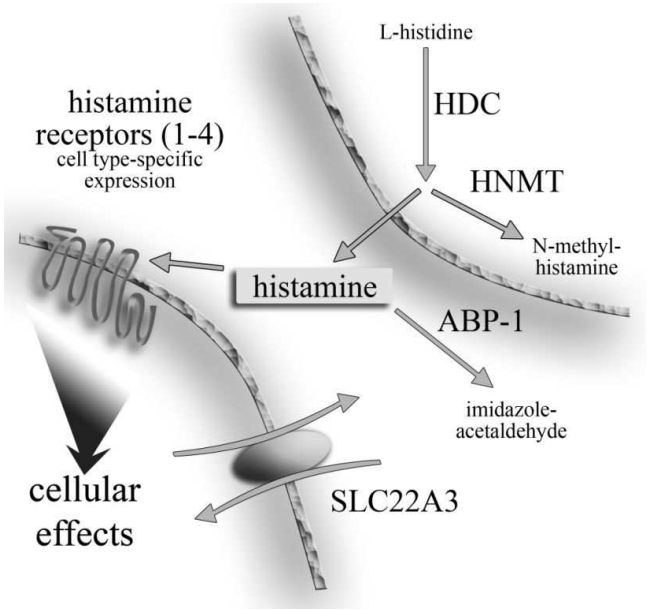


Figure 3.

Schematic figure of histamine metabolism and activity

Histamine synthesized from L-histidine in different cell types may act on four different HRs (H_1R-H_4R). Transporters (e.g. SLC22A3) enable its passage through cell membranes. The main histamine degrading enzymes (HNMT and ABP1) are also influence the local level of the amine.

HRs, overview of the biological effects of histamine

The diverse physiological and pathological effects of histamine are mediated by four HRs, all being typical heptahelical proteins the activation of which is mediated by G proteins. The first histamine receptor discovered (H_1R) possesses the lowest binding affinity to the amine and is linked mainly to allergic reactions. It shares the typical features of G-protein-coupled receptors activating Gαq. The ligand binding to H_1R leads to increased cytosolic Ca^{2+} and cAMP levels and results in the activation of NFκB pathway. In the lung it mediates bronchoconstriction and increased vascular permeability. The H_1R is expressed in numerous cell types, including airway and vascular smooth muscle cells, hepatocytes, chondrocytes, nerve cells, endothelial cells and some immune cells [37] [38].

Histamine receptor 2 (H_2R) is coupled to the adenylate cyclase and phosphoinositide second messenger systems by separate GTP-dependent mechanisms. Receptor binding also activates c-Fos, c-Jun and protein kinase C. The ligand binding affinity of H_2R exceeds that of the H_1R and similarly to H_1R, H_2R is also expressed in various cell types. Initially it was thought to regulate a limited number of activities such as heart contraction and gastric acid secretion however, it is now quite clear that various regulatory functions of histamine during cell proliferation, differentiation and immune response are exerted through H_2R activation [37] [38].

Histamine receptor 3 (H_3R) is predominantly expressed in the central nervous system and is involved in the regulation of the adjustment of locomotor activity and circadian rhythm of the body.

In 2001, several working groups suspected the existence of a fourth HR based on *in silico* studies and validated by functional studies [44] [45]. Mouse and human H_4R exhibit 68% sequence identity [46] and despite some differences H_4R is typically expressed by cells of the immune system, preferentially peripheral hematopoietic cells including eosinophils, basophil granulocytes, mast cells, T and B lymphocytes and DCs [43] [47]. However, the presence of H_4R has recently been shown in tissues functionally distant from the immune system, like in the brain or liver [43] [48]. As H_4R is widely distributed in immune cells its role in inflammation, hematopoiesis and directing immune responses was suspected.

Although the picture is not complete it become evident that it had an impact on the chemotaxis of several immune cells (mast cell, eosinophil granulocyte, DC), regulated cytokine production of various cells and had a role in allergy, inflammatory processes and autoimmune diseases [47] (Figure 4.).

Figure 4.

Summary of the effects of H_4R

The presence of H_4R mostly in the immune system and its immunomodulatory role in cytokine production argue for the pathophysiological significance of the receptor in inflammatory conditions that are characterized by increases in immune cell numbers, such as asthma, allergic disorders and autoimmune diseases [47].

The effects of H_4R are mediated through the activation of Gi/o proteins. As a result of a multistep signaling cascade Ca^{2+} flows into the cell, cAMP level is decreasing and the MAPK pathway is activated [37]. The ligand binding affinity of H_4R is greater than that of H_1R or H_2R, but lower than that of the H_3R. The growing number of specific agonists and antagonists holds promise of novel tools in the therapy of inflammatory and/or autoimmune disorders [47]. Among the commercially available agonists 4-methylhistamine (4-MH) can be mentioned, which has long been known as a H_2R agonist, however after the discovery of H_4R it emerged as a high affinity ligand of H_4R with 100 times more efficient binding than other HRs [49]. The antagonists JNJ7777120 and JNJ10191584 [50] [51] [52] are different in a single nitrogen atom and both bind to H_4R with high affinity (Ki=4 nM and Ki=27 nM).

HRs are remarkably different in their ligand binding affinity, cell surface expression, tissue distribution, the signaling pathways they use and functional features [38] [43] [53]. Currently, synthetic ligands for all HRs are also available and their number is increasing. These compounds act either as specific antagonists, which block the function of one receptor

or may interfere with the signaling of more than one receptor. Antagonists show high structural similarity to histamine and can bind very strongly to HRs however, their binding didn't result in receptor-mediated signaling. On the contrary, receptor agonists activate the receptor upon their binding, signal transduction is induced and biological responses are provoked. HRs may be present on the cell surface in active or inactive forms and in steady states these two activation states are in constant equilibrium; agonist binding stabilizes the active conformation, while antagonists stabilize the inactive state of the receptor [54]. The distribution, functional role and typical ligands of the various HRs are summarized in Table 1.

histamine receptor	tissue expression	intracellular signaling molecules	typical G protein	agonists	antagonists
H_1R	nerve cell, airway and vascular smooth muscle cell, hepatocyte, chondrocyte, endothelial cell, neutrophil, eosinophil granulocyte, monocyte, DC, T and B cell	Ca^{2+} cGMP, phospholipase D, phospholipase A_2, NFκB	Gq	histaprodifene betahistine histamine-trifluoromethyl-toluidide dimaleate	mepyramine chlorpheniramine cetirizine astemizole clemastine terfenadine loratadine pyrilamine clozapine triprolidine diphenhydramine
H_2R	nerve cell, airway and vascular smooth muscle cell, hepatocyte, chondrocyte, endothelial cell, epithelial cell, neutrophil, eosinophil granulocyte, monocyte, DC, T and B cell	adenilate-cyclase, cAMP, c-Fos, c-Jun, PKC, p70S6K	Gs	dimaprit amthamine impromidine	zolantidine cimetidine ranitidine tiotidine famotidine
H_3R	histaminerg neuron, eosinophil granulocyte, DC, monocyte, low expression in peripheral tissues	high Ca^{2+}, MAP kinase, cAMP inhibition	Gi/o	imetit R-α-methylhistamine immepip	thioperamide clobenpropit carboperamide iodoproxyfan ciproxifan
H_4R	hematopoetic cells, mast cell, eosinophil, neutrophil, basophil granulocyte, DC, T cell, nerve cell, hepatocyte	high Ca^{2+}, cAMP inhibition	Gi/o	clobenpropit imetit clozapine 4-methyl-histamine	thioperamide JNJ7777120 JNJ10191584 JNJ77771202

Table 1.

Summary of the most important features of HRs

The table displays the tissue expression and the most relevant intracellular signaling molecules of the four types of HRs (H_1R-H_4R). The most important ligands (agonists and antagonists) used in receptor blocking studies are shown as well.

3.3. Effects of histamine on DCs

Histamine synthesis and the expression of HRs by DCs

While circulating in the body, DCs are under the regulatory effects of different chemokines, cytokines, inflammatory mediators or even low molecular mass histamine. It is well-known that HDC, the only histamine synthesizing enzyme, is expressed both at RNA and protein levels in human DCs differentiated *in vitro* from peripheral blood monocytes. Histamine itself has also been detected in DCs intracellularly [9], however to date, the release of newly synthesized histamine (also referred to as ”nascent histamine”) has been shown only in murine [55] [56] and not in human DCs.

The expression of H_1R, H_2R and H_4R was observed by many working groups [56] [57] [58] [59], while the detection of H_3R is controversial [60] [61]. Interestingly, neither H_1R nor H_2R was found on the surface of either *in vitro* differentiated LCs or those isolated from human epidermis. This was probably due to the effects of TGF-beta 1 specifically applied for LC differentiation [62]. Recently the expression of the lastly discovered H_4R by both *in vitro* generated monocyte-derived LCs (mRNA and protein) and primary LCs from both murine and human skin samples was documented [63] [64].

It was proven that distinct DC subtypes differ not only in their functions served in immune processes but also in their surface molecule pattern [62]. Observations proving that DCs express both HRs and their ligand support the concept that DCs may be under the influence of histamine derived not only from professional histamine synthesizing cells, but also from DCs themselves.

Effect of histamine on in vitro differentiation and maturation of DCs

Differentiation of DCs is a complex, highly regulated process accompanied by phenotypical changes and the alteration in the expression of various molecules. Both HDC protein expression and intracellular histamine content were found to be increased during cytokine-induced *in vitro* differentiation of DCs from human peripheral blood monocytes. In parallel with these changes, the expression of functionally relevant cell surface molecules (CD80, CD86, CD40, CD45 and CD11c) was elevated, too. Some of these effects could be effectively inhibited by the blockade of *de novo* histamine production, inhibition of histamine

binding or intracellular interaction of histamine with cytochrome P-450 moieties. All these data indicate a substantial role of histamine in *in vitro* DC differentiation [9]. Similar inhibitory effect of histamine has been detected on CD1a expression during *in vitro* DC differentiation that was antagonized by a H_2R antagonist [65]. After differentiation, a variety of factors of viral or bacterial origin (such as LPS or cytokines like IFNγ, IL-1ß, TNF-α) may induce terminal maturation of DCs. However, no effect of histamine was detected in LPS driven maturation of immature monocyte-derived DCs [66].

Endocytosis

Immature DCs (IDCs) exhibit extraordinary ability to sample their surrounding environment. They utilize different receptors and mechanisms for antigen capture such as phagocytosis of particles, macropinocytosis which occurs constitutively in DCs and allows continuous internalization of antigens present in the fluid phase, and receptor-mediated endocytosis which involves the internalization of soluble antigens after clustering of receptors in clathrin-coated pits.

After histamine challenge, an elevated fluorescein isothiocyanate-labeled (FITC) latex bead phagocytosis was observed by human monocyte-derived DCs [65]. Other authors reported that phagocytosis of FITC-ovalbumin-coated (OVA) latex beads by murine DCs was not stimulated by histamine [56], their results showed that histamine was able to increase the endocytosis of soluble HRP (horseradish peroxidase) and FITC-OVA. Enhancement of endocytosis was completely suppressed by a H_2R antagonist, but not with H_1R and H_3R/H_4R antagonists. One may conclude that the ability of histamine to increase antigen uptake depends on the form of antigen and/or on the mechanism of antigen internalization by DCs [56].

DC migration

Migration is an important and indispensable feature of DCs. IDCs migrate to both body surfaces and interstitial spaces where they easily make contact with self- or foreign antigens. After antigenic challenge, migration of DCs is a prerequisite to activate T cells and thus, link innate and adaptive immune responses.

In human monocyte-derived IDCs (but not in mature DCs (MDCs)) histamine, H_1R and H_3R agonists induce intracellular Ca^{2+} mobilization and F-actin polymerization in a dose-dependent manner [60]. Damaj *et al.* also found that histamine mobilized intracellular Ca^{2+} in human monocyte-derived DCs that was inhibited by a H_3R/H_4R antagonist. Another study

demonstrated similar effects of histamine regarding actin polymerization in IDCs, however, the data on the receptors involved were controversial. According to Gutzmer *et al.* H_2R and H_4R agonists trigger F-actin polymerization, an effect that can be antagonized by specific receptor antagonists [58] [61].

Several independent research groups established that histamine [59] [61] or HR agonists [60] [61] are strong chemotaxins for IDCs. In contrast, they are ineffective for MDCs suggested by the observation that MDCs did not migrate in response to histamine challenge [60]. By using specific receptor antagonists the involvement of both H_3R [59] [60] and H_1R [60] has been shown in the modulation of DC migration. *In vivo* studies revealed that LC migration to draining lymph nodes was modulated by mast cell-derived histamine, and this effect was blocked by a H_2R antagonist [67]. Mouse DC migration was impaired by a H_4R antagonist; upon *in vivo* administration of JNJ7777120 reduced number of labelled DCs was detected in lymph nodes [68]. Histamine via H_2R, also promotes peptidoglycan-induced accumulation of DC subsets in lymph nodes [69]. In a skin DC migration assay both histamine and a H_4R agonist induced an enhanced chemotaxis which was blocked by H_1R and H_4R antagonists. These results were confirmed by *in vitro* migration experiments using bone marrow-derived mouse DCs [70].

Thus, based on both *in vivo* and *in vitro* data, histamine is able to modulate DC migration via different receptors.

Antigen presentation

Even though an increasing body of evidence supports that histamine has an impact on antigen presentation, this essential immunological process needs further investigation, especially because most results are obtained from murine experiments.

Expression of costimulatory and accessory molecules (CD40, CD80, CD86) and MHC class II, involved in effective antigen presentation, are enhanced by histamine transiently on human monocyte-derived IDCs. Furthermore, H_1R and H_2R antagonists prevented histamine-induced CD86 expression [57]. Mazzoni also reported a moderate, but consistent increase in CD86, CD80 and MHC class II expression however, histamine had no effect on the expression of CD40 [66].

Splenic DCs of $HDC^{-/-}$ mice kept under histamine-free conditions, displayed a more efficient *in vitro* antigen presentation as compared to cells from WT mice. This difference was not a result of an altered distribution of DCs between or within the major functional sub-

populations or to major changes in the costimulatory molecule profile (e.g. CD40, CD80, CD86) [71].

Cross-presentation is the process by which extracellular antigens (normally presented in association with MHC class II molecules), are routed for presentation on MHC class I molecules, enabling extracellular antigens to activate $CD8^+$ T cells. This pathway may lead either to tolerize or activate of antigen-specific $CD8^+$ T cells. Histamine markedly improved cross-presentation of soluble OVA, but not that of OVA-coated latex beads in mouse IDCs. Thus, it seems that the ability of histamine to increase cross-presentation is dependent on the form of the antigen and/or the mechanisms through which the antigen is internalized by DCs, since cross-presentation of the pinocytosed but not of the phagocytosed OVA was facilitated by histamine. Interestingly, the expression of MHC II, but not that of MHC I was increased after histamine treatment, and the stimulation of cross-presentation was prevented by H_3R/H_4R antagonists [56].

T cell polarization

DCs are professional antigen presenting cells, which effectively prime naive antigen-specific T cells. Based on their ability to direct Th1 or Th2 differentiation, MDCs fall into the categories of either dendritic cell 1 (DC1) or dendritic cell 2 (DC2) phenotypes, which in turn, facilitate the development of Th1 and Th2 cells, respectively. DC maturation is associated with the synthesis of numerous cytokines and chemokines that act on T cell polarization. The most intensively studied cytokines include the Th1 type IL-12 and the Th2 or T regulatory type IL-10.

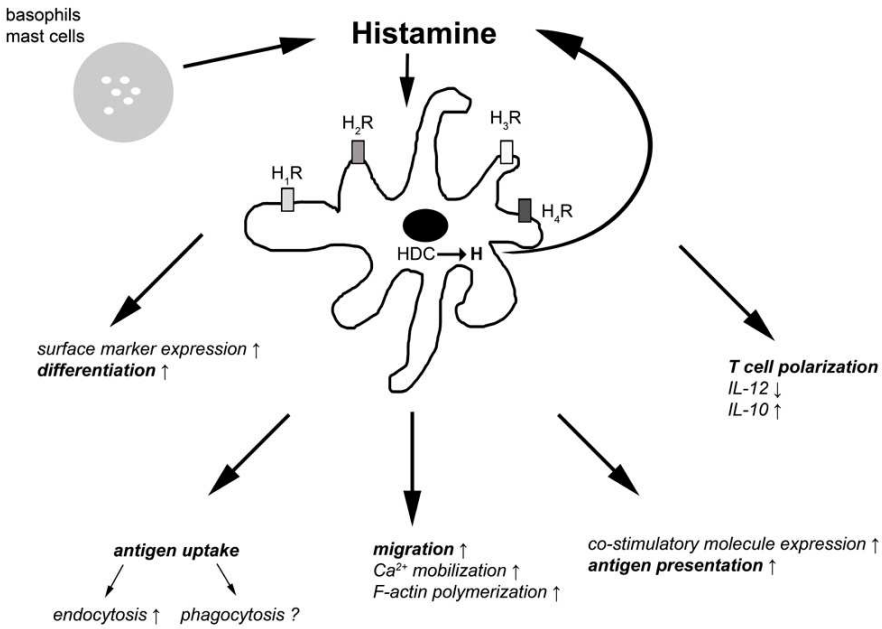


Figure 5.

Summary of histamine effects on DC functions

Arrows indicate the source of histamine (H), the crucial impacts and their direction (decrease or increase) on DC functions via different HRs (H_1R-H_4R).

The majority of experimental data support that histamine induces an altered cytokine expression profile in DCs, and favor Th2 polarization. This effect is exhibited even in the presence of IFNγ, a strong DC1 promoting factor [57]. Histamine inhibited LPS- or IFNγ-induced IL-12 responses and the production of some other pro-inflammatory cytokines such as IL-1ß and IL-6. This effect could be antagonized by both H_1 and H_2 receptor antagonists [57] [58] [66]. Others demonstrated the involvement of both H_2R and H_3R but not H_1R in histamine-induced suppression of IL-12 production. In contrast, relevant Th2 cytokines and LPS-stimulated IL-8 and IL-10 synthesis, were significantly increased in histamine-treated human monocyte-derived MDCs [60] [66].

After the discovery of H_4R, the possible involvement of this receptor in Th polarization was studied extensively. It was demonstrated that the reduced production of the paramount Th1 cytokine, IL-12p70 was mediated not only by H_2R but also by H_4R [61]. *In vitro* studies indicated that in DCs from $H_4R^{-/-}$ mice and upon the blockade of H_4R of mouse splenic DCs, cytokine (IL-6) and chemokine production (KC, MIP-1 alpha) were significantly

decreased. Furthermore, the ability of H_4R-deficient DCs to induce Th2 responses in T cells is limited [55].

The migration of Th cells into target organs is regulated by different sets of chemokines. Histamine was shown to upregulate the production of Th2-attracting chemokines CCL17 and CCL22 and to downregulate IFNγ-induced CXCL10 production by human monocyte-derived IDCs [72].

These data indicate that histamine is involved in Th2 polarization, it downregulates IL-12 secretion and stimulates the synthesis of certain Th2-attracting chemokines and IL-10 by DCs (Figure 5.).

4. AIMS OF THE STUDIES

During their life cycle DCs are often get exposed to histamine. This multifunctional amine is known to influence DC differentiation and is involved in the regulation of various DC functions. The activities of DCs may be associated with more than one HR, as shown by the role of H_1R and H_2R in the functional activities of these cells [38] [73]. However, after the discovery of the novel H_4R, some previous results await for re-evaluated. Thus, in our two experimental systems first we tested whether histamine had an effect on the functional activities of mouse DCs. The migration, adhesion, cytokine production and the antigen presenting function of DCs were investigated. Beyond these studies we also investigated the possible impact of the lastly discovered H_4R in mediating these histamine effects. A gene knock out (KO) mouse model and *in vitro* receptor blocking were used to answer these questions.

<u>Specific questions addressed in the murine system:</u>

- Comparison of HR expression in splenic and bone marrow-derived DCs
- Investigation of the effect of histamine on *in vitro* antigen presentation
- Testing the role of H_4R in histamine-modulated antigen presentation in WT and $H_4R^{-/-}$ DCs
- Testing the role of histamine and H_4R in DC migration and adhesion
- The role of H_4R in the cytokine production of *in vitro* activated DCs

- Comparison of cytokine production of DCs isolated from WT and $H_4R^{-/-}$ mice following *in vivo* activation

It is also known that $CD1a^-$ and $CD1a^+$ DCs derived from human monocytes exhibit distinct functional characteristics being the $CD1a^+$ subset more inflammatory as compared to its $CD1a^-$ counterpart. The cytokine, chemokine production and phagocytic function of the two subpopulations are also different [74]. We postulated that histamine could affect (perhaps differently) the activities of the two subsets and the results would enable us in better understanding the mechanism of histamine on DCs and further the diseases in which these cells were involved. Therefore, in the second set of experiments we tested how histamine influences the development and functional activities of $CD1a^-$ and $CD1a^+$ DC subsets differentiated *in vitro* from human $CD14^+$ blood monocytes. We also tested the HR dependence of the identified effects.

<u>Specific questions addressed in the human system:</u>

- Comparison of HR expression in the functionally distinct $CD1a^-$ and $CD1a^+$ DC subpopulations derived from human blood monocytes
- Testing the effect of histamine on the differentiation of DC subsets
- Investigation of the modulatory role of histamine on $CD1a^-$ and $CD1a^+$ DC activation
- Impact of histamine on DC cytokine and chemokine production of DCs
- Testing the effect of histamine on *in vitro* DC migration by using specific receptor blockers. Identification of the receptor responsible for the modifying effect of histamine on DC functions.

5. MATERIALS AND METHODS

Mouse studies

1. Animals

$H_4R^{-/-}$ mice were generated by Lexon Genetics, and were kindly provided by Dr. Robin L. Thurmond (Johnson & Johnson Pharmaceutical Research and Development, San Diego, CA, USA). Mice were crossed on to BALB/c background for seven generations in our laboratory. Homozygous wild-type controls (WT) of the same backcross number and generation were used as controls. For all experiments, mice were maintained under specific pathogen-free conditions, at constant temperature (22°C) and under a light cycle of 12 hours light/12 hours darkness in groups of 10. Food and water were freely available. Animals were used at 8–12 weeks of age. All experiments were carried out with the approval of the local ethics committee. The authors adhered to the Declaration of Helsinki and the IASP for the care and use of animals throughout the study.

2. Primary cells

DC isolation

DCs were purified from spleens of WT and $H_4R^{-/-}$ mice after collagenase D digestion (2 mg/ml in RPMI supplemented with antibiotics) (Roche Diagnostics, Mannheim, Germany). $CD11c^+$ cells were obtained by CD11c-MACS immunomagnetic bead selection (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Briefly, spleens were put into Petri dishes and injected with collagenase D solution, cut into small pieces and incubated for 30 min at 37ºC. Cell suspension was filtered (40 µm filter), washed with MACS buffer (1xPBS+0.5% BSA+(Sigma-Aldrich, Deisenhofen, Germany) 2 mM EDTA) and the final concentration was set to 10^8 cells/400 µl MACS buffer. Then 5 µl anti-mouse CD16/CD32 (FcγIII/II receptor blocking agent) monoclonal antibody (BD Biosciences PharMingen, San Diego, CA, USA) was added to the cells (5 min; 4ºC) to minimize aspecific binding. After addition of CD11c microbeads (10^8 cells/100 µl) cells were incubated for 15 min at 4ºC, washed and cell numbers were set to 10^8 cells/500 µl MACS buffer. MS/LS columns (Miltenyi Biotec) were used for magnetic cell separation. After binding of $CD11c^+$ cells, the column was washed 3 times and the cells were eluted with 1 ml/3 ml MACS buffer. With this procedure the average yield was ~ $2x10^6$ cells from a spleen. The homogeneity of

the cell population was controlled by flow cytometry, the frequency of $CD11c^+$ DCs was ~ 95% in all experiments (Figure 6.).

For setting up short term *in vitro* cultures, DCs were put into RPMI-1640 medium (Sigma-Aldrich) supplemented with:

- 10% heat-inactivated FCS (Invitrogen, Gibco, Paisley, USA)
- Penicillin (50 U/ml, Invitrogen)
- Streptomycin (50 µg/ml, Invitrogen)
- L-glutamine (2 mM, Invitrogen)
- 2-mercaptoethanol (200 µM, Sigma-Aldrich)

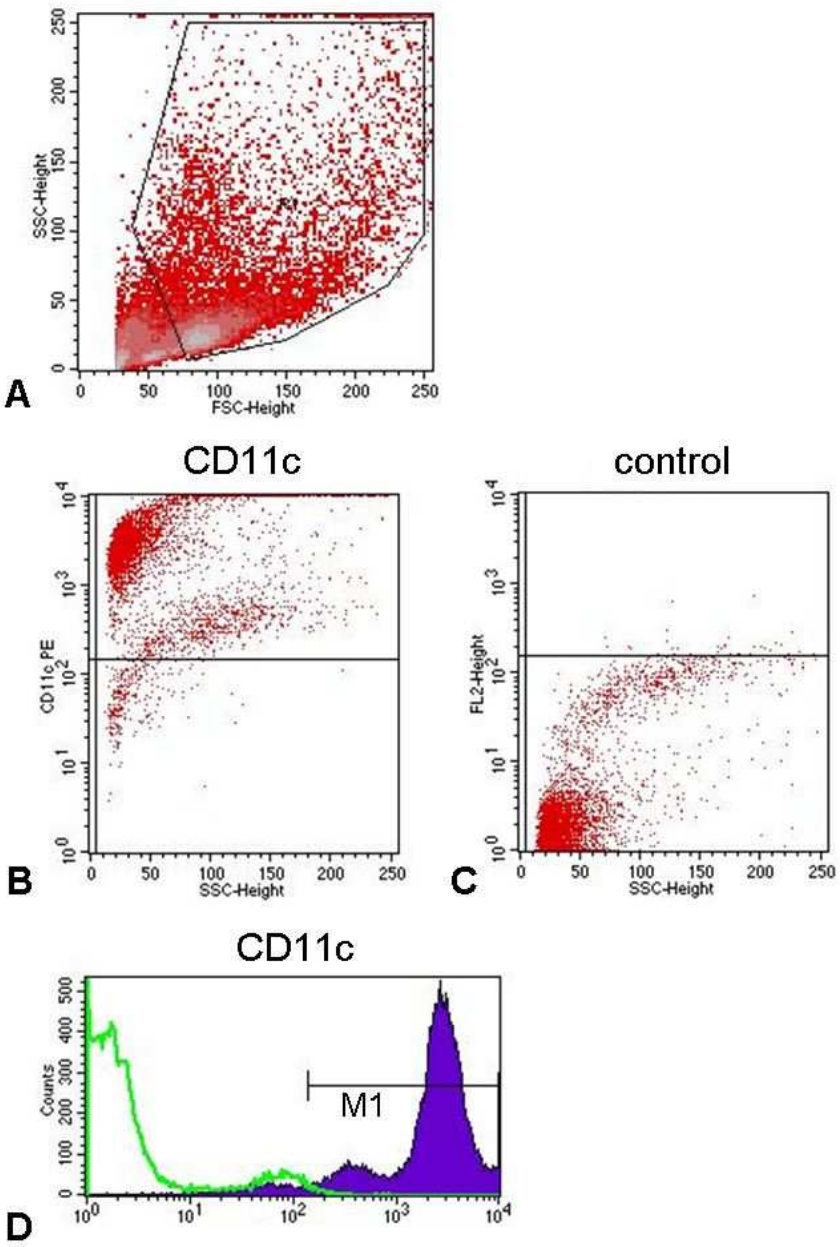


Figure 6.

Distribution of $CD11c^+$ DCs after magnetic cell separation

Detection of CD11c expression was assessed by flow cytometry after separation of spleen-derived DCs. Based on the forward and side scatter (FSC-SSC) dot plots (A) DCs were identified by the cell surface expression of CD11c within the morphologically viable cell population (B). Figure C shows a representative dot plot without staining of the cells and D is a histogram of $CD11c^+$ morphologically viable cells. Green line and the M1 marker represent control staining and the difference between the control and specific CD11c staining intensities, respectively.

In vitro differentiated mouse DCs

For DC differentiation the procedure of Lutz *et al.* [75] was used. Briefly, bone marrow was flushed from tibiae of the mice with PBS. Clusters within the suspension were disintegrated by vigorous pipetting. After washing the cells, $2x10^6$ cells in RPMI-1640 medium supplemented with Penicillin (100 U/ml), Streptomycin (100 µg/ml), L-glutamine (2 mM), 2-mercaptoethanol (50 µM) and 10% heat-inactivated FCS and 200 U/ml recombinant mouse (rm) GM-CSF (R&D Systems Inc. Minneapolis, USA) were transferred to bacteriological Petri dishes with 100 mm diameter and incubated at 37 ºC. At day 3 another 10 ml medium containing 200 U/ml rmGM-CSF was added to the plates. At days 6 and 8 half of the culture supernatant was collected and centrifuged. The cell pellet was resuspended in 10 ml fresh medium with rmGM-CSF and put back into the original plate. At day 10 the differentiated cells were used for the *in vitro* experiments. To reduce granulocyte contamination in some experiments culturing of cells was continued until 13 days in the presence of lower (30-100 U/ml) amounts of rmGM-CSF. The ratio of $CD11c^+$ DCs varied between 85%-95% as controlled by flow cytometry.

3. Phenotypic characterization of DCs

The expression of HRs (H_1R, H_2R, H_4R) and the relevant DC markers (CD11c, CD11b, MHCII, CD40, CD80, CD86) was measured by flow cytometry (Table 2.). After washing the cells in 1% BSA+PBS, they were stained with 0.5 µg/µl antibody in 50 µl 1% BSA+PBS solution, incubated 20 min in dark, washed again and fixed in 200 µl 2% PFA solution (Sigma-Aldrich). Part of the cells (splenic or differentiated DCs) was treated with histamine (1 µM and 10 µM).

The length of histamine incubation of splenic DCs (in the presence of rmGM-CSF) was 4 or 19 hours, whereas bone marrow-derived DCs were treated on day 13 with histamine for 24 hours.

For detection of HR expression, the cells were first labelled with CD11c-specific antibody for 20 min, washed, fixed and permeabilized with 2 ml Saponin-PBS (Sigma-Aldrich) and immediately centrifuged. HR-specific antibodies (H_1R 1 µl; H_2R 2 µl; or H_4R 1 µl) were added to the pellets and incubated for 20 min, washed and stained with biotinylated anti-rabbit Ig (1 µl). Finally, streptavidin-APC staining was used to detect the receptor proteins. Fluorescence intensities of samples were detected by FACS Calibur™ (BD Biosciences). 1 µg/ml lipopolysaccharide (LPS, Sigma-Aldrich) was used to activate DCs at 37ºC for 24 hours.

Flow Cytometry	*Antibody*	*Manufacturer*
CD11c	PE Hamster Anti-Mouse CD11c	BD Biosciences PharMingen
CD11b	FITC anti-mouse CD11b	BD Biosciences PharMingen
CD40	FITC anti-mouse CD40	BD Biosciences PharMingen
CD80	FITC anti-mouse CD80	BD Biosciences PharMingen
CD86	FITC anti-mouse CD86	BD Biosciences PharMingen
MHCII	FITC anti-mouse I-A/I-E	BD Biosciences PharMingen
H_1R	Rabbit Anti-Rat H_1R IgG	Alpha Diagnostic
H_2R	Rabbit Anti-Rat H_2R IgG	Alpha Diagnostic
H_4R	Rabbit Anti-Mouse (Rat) IgG	Santa Cruz Biotechnology
secondary antibody	Anti Rabbit Ig biotinilated from donkey	Amersham International
terciary antibody	Streptavidin APC	BD Biosciences PharMingen

Table 2.

Specification of antibodies used for flow cytometric measurements

The table summarizes the labelling, isotype, source and commercial availability of the antibodies applied in flow cytometric measurements.

4. *In vitro* antigen presentation and IL-2 production

Freshly isolated mouse splenic DCs ($2x10^3$ cells/well) were co-cultured with the 5/4E8 T cell hybridoma cell line ($2x10^4$ cells/well) , specific for the human aggrecan G peptide [76] for 24 hours at 37ºC. Wells contained 200 µl culture medium and 1 µg/ml human aggrecan G peptide (ATEGRVRVNSAYQDK) (kindly provided by Szilvia Bősze and Ferenc Hudecz, Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eötvös Loránd University, Budapest, Hungary). In technical control wells no peptide was added. 24 hours later IL-2, produced by the activated 5/4E8 hybridoma cells, was measured by sandwich ELISA (R&D Systems Inc.) according to the manufacturer's instructions. DCs were challenged with different concentrations (0.1 µM-1000 µM) of histamine (Sigma-Aldrich), or 4-methylhistamine (4-MH, 1-100 µM, Tocris Bioscience, Ellisville, USA) administered together with the aggrecan peptide. In some experiments HR antagonists, famotidine (H_2R ligand, 10 µM, Sigma-Aldrich) and JNJ10191584 (H_4R ligand, 10 µM, Tocris Bioscience) were applied 1 hour before histamine (1 µM) treatment in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich) supplemented with:

- Penicillin (50 U/ml)
- Streptomycin (50 µg/ml)
- L-glutamine (2 mM)
- 2-mercaptoethanol (50 µM)
- 10% heat-inactivated FCS
- 1% non-essential amino acids (Sigma-Aldrich)
- 1% sodium pyruvate (Sigma-Aldrich) was used in all antigen presentation experiments.

5. *In vitro* migration assay

Migratory response of WT and $H_4R^{-/-}$ DCs was examined in 24 well Transwell plates (Corning Incorporated/Costar, New York, USA) of 5 µm-pore-size polycarbonate filters. The Transwell system was preincubated for 1 hour before the migration assay, with 600 µl and 200 µl medium (complete RPMI-1640) in the lower and the upper chambers, respectively. Then the upper chamber with the membrane was filled with 200 µl of cell suspension ($1x10^6$ cells/ml) in RPMI-1640 and 600 µl of medium with or without histamine (1, 10, 100 µM) or 4-MH (1, 10, 50 µM) was added to the lower chambers. Control wells contained only RPMI medium. Migration was allowed for 2 hours at 37 ºC. Transwells were removed and $2x10^4$ polystyrene microparticles (diameter of 15 µm, Fluka Chemie GmbH, Buchs, Sweden) were

put into the lower chamber. The number of transmigrated cells in the bottom chamber was counted by flow cytometry normalized to polystyrene microparticles.

In the experiments using differentiated DCs 1 μM histamine was added to the cells on days 0, 3, 6 and 8 of the differentiation process. Migration of 10-day bone marrow-derived DCs of WT and $H_4R^{-/-}$ was compared by using the same experimental protocol.

6. Cell adhesion assay

Adhesion characteristics of DCs were determined with an impedance-based method by using the xCELLigence RTCA SP system (Roche Applied Science, Indianapolis, IN, USA) [77]. Theoretical background of the measurement is that viable cells are good insulators. Therefore, adhesion of cells to small reference electrodes, connected to AC circuits, real-time increases the impedance and this value correlates with the cell numbers attached [78]. The main advantage of this method is that it enables real-time detection of adhesion. Bottom areas of wells in E-plates®96 (Acea, San Diego, CA, USA) served as gold electrode furnished surfaces of the measurements. Prior to the experiments the electrodes were coated with 25 μl 0.025% human plasma fibronectin (FC010 Millipore, Temecula, CA, USA) containing 0.1% gelatin solution (Sigma-Aldrich) for 20 min at 4 °C. In the next step the coating solution was carefully removed from the wells, and 100 μl complete medium (RPMI-1640) was added as a reference substance of baseline measurements. The baseline impedance was detected in tissue culture incubator (37 °C, 5% CO_2) for 1.5-2 hours. DCs were gently removed and suspended in 100 μl culture media, quantified, and seeded into wells (50.000 cells/well of E-plate in triplicate). Values of impedance (Z) were recorded in a real time mode at 10 kHz for 12 hours (sampling frequency of data collection was 1/15 sec). Impedance was represented by the cell index (CI) values ($(Z_n-Z_0)/F_i$ [Ohm]/15[Ohm], Z_0: background resistance, Z_n: individual time point resistance, F_i: constant characteristic for frequency) and the delta cell index (ΔCI) was calculated for the baseline. CI data were analyzed and the slope of the curve was calculated by RTCA software v1.2 (Roche Applied Science). The software enables the evaluation of several parallels by simplifying the mathematics. Although its value is changing by time, the slope for any time interval can be calculated, even if the CI value does not change. The program considers the starting and the end points and the value of the slope is always lower that the slope of a fitted line. By this recommended mode of evaluation we can not associate a biological function to a numerical, exact slope value, but we are able to indicate the relative speed of adhesion and indicate when it is faster or slower. In the measurements we evaluated

CI values at 2 hours beside CI time dependence and in the first 1.5 hour slope analysis was performed by RTCA software as seen in Figure 7.

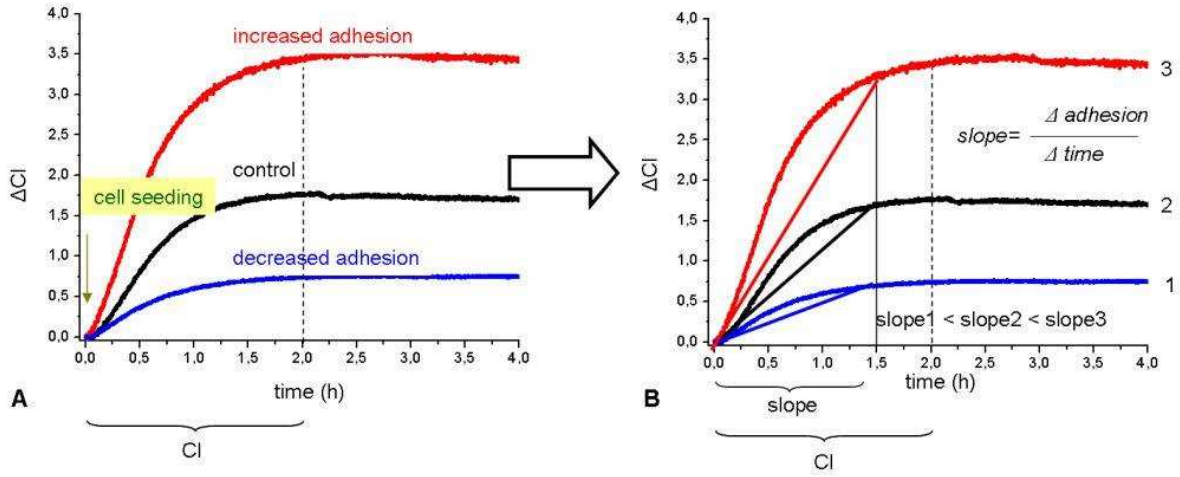


Figure 7.

Measurement of cell adhesion

Typical adhesion curves of DCs (A) and determination of the slopes of CI curves are demonstrated (B) on the figure. Adhesion has determined phases. After seeding of the cells a fast increase is detectable in CI values, then when they are adhered to the surface reach a stable state. Evaluation of the curves can be performed by two methods: 1. comparing CI values in a determined time point after complete adherence of the cells or 2. calculating and comparing the slopes of the CI curves in a chosen time period by performing a linear-line fit.

7. *In vitro* DC stimulation

Spleen-derived $CD11c^+$ DCs were plated to 12 well plates at a density of $3x10^6$ cells/well in complete RPMI-1640 medium. With the exception of the control wells, DCs were incubated with 1 µg/ml LPS for 24 hours at 37 ºC. Before LPS stimulation, cells were pretreated either with a H_4R agonist (4MH, 0,1 µM) alone for 15 min or with a H_4R antagonist (JNJ7777120, 10 µM, Sigma-Aldrich) for 15 min prior to the agonist treatment. After incubation the cells were processed for RNA isolation and subsequent real-time PCR analysis.

8. *In vivo* DC stimulation

WT and $H_4R^{-/-}$ mice (6 animals in each group) were injected intra-peritoneally with either 200 µl CFA emulsion (Sigma-Aldrich) containing 0.1 mg of Mycobacterium tuberculosis H37 Ra or 200 µl PBS. After 7 days of immunization, mice were sacrificed and spleens were removed for DC isolation. The obtained $CD11c^+$ DCs were used for RNA preparation followed by reverse transcription and quantitative PCR analysis.

9. RNA isolation

RNA isolation was performed with RNeasy® Mini Kit (Qiagen, Duesseldorf, Germany) according to the manufacturer's instructions. During this protocol samples were digested with the mixture of 10 µl DNase I solution+70 µl RDD buffer (Qiagen) and incubated for 15 min. Purity of RNAs was determined with NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) on UV 260/280 nm.

10. Reverse transcription

2 µg total RNA/sample was reverse transcribed using:

- 4 µl 10x buffer (Mg^{2+} free, Promega Corp. Madison, WI, USA)
- 8 µl 25 mM $MgCl_2$ (Promega)
- 4 µl 10 mM dNTP mix (100mM each, Promega)
- 2 µl RNasin Ribonuclease Inhibitor (Promega)
- 1 µl Random primers (Promega)
- 1 µl MuLV reverse transcriptase (Applied Biosystems, Foster City, CA, USA)
- H_2O for 40 µl

11. Quantitative real-time PCR

Relative quantification of target mRNA was performed with a TaqMan real-time RT-PCR assay on an ABI Prism 7000 Sequence Detection System (Applied Biosystems) following the manufacturer's instructions. Taqman probe sets were used as follows: TNF-α, IL-1ß, IL-6, IL-4, IL-10, Gata-3, IL-12, IFNγ, T-bet, CCR7, CCL19 and GAPDH as housekeeping internal standard/control. All probe sets were purchased from Applied Biosystems. Wells of 96 well plates (Thermo Scientific) were filled with the mixture of 7.5 µl Taqman Universal PCR Master Mix (Applied Biosystems), 0.76 µl probe, 1.2 µl cDNA and 5.52 µl H_2O (15 µl/well). GAPDH-normalized signal levels were calculated using the comparative C_t method.

Gene symbol	*Assay code*
TNF-α	Mm00443258_m1
IL-1ß	Mm00434228_m1
IL-6	Mm00446190_m1
IL-4	Mm00445259_m1
IL-10	Mm00439616_m1
Gata-3	Mm00484683_m1
IL-12	Mm00434165_m1
IFNγ	Mm00801778_m1
T-bet	Mm00450960_m1
CCR7	Mm00432608_m1
CCL19	Mm00839967_g1
GAPDH	Mm99999915_g1

Table 3.

Quantitative real-time PCR primers and probes

The table summarizes the specifications of the primers applied in quantitative real-time PCR measurements.

12. Western blot analysis

Cells were washed in 1xPBS and then lysed in ProteoJET Mammalian Cell Lysis Reagent (Fermentas GmbH, Sankt Leon-Rot Germany) supplemented with Aprotinin From Bovine Lung (Sigma-Aldrich), Leupeptin trifluoro-acetate salt (Sigma-Aldrich), Phenylmethanesulfonyl fluoride (PMSF, Sigma-Aldrich) and Sodium orthovanadate (Na_3VO_4). The samples contained 15 µg protein and were incubated (5 min, 100ºC) in 4x loading buffer (30% glycerin, 10% ß mercaptoethanol, 0.7 M SDS, 0.25 M TRIS-HCl and 0.0001% bromophenol blue) and the protein bands were separated by electrophoresis (Mini-Cell electrophoretic system (BioRad Laboratories, Hercules, CA, USA) in 10% Bis-Tris polyacrylamide gel (Lonza, Basel, Switzerland) and then transferred to PVDF membrane (BioRad Laboratories). After blocking with 4% BSA (1 hour), membranes were washed and probed with primary antibodies (Table 4.). The Ag-Ab complexes were labelled with appropriate HRP-conjugated secondary antibodies and visualized by ECL Plus Western Blotting Detection Reagents (GE Healthcare, Buckinghamshire, UK) on x-ray film (Retina XBM film Fotochemische Werke GmbH, Berlin, Germany).

Western blot	*Antibody*	*Manufacturer*	*Applied dilution*
H_1R	Rabbit Anti-Rat H_1R IgG	Alpha Diagnostic	1:2000
H_2R	Rabbit Anti-Rat H_2R IgG	Alpha Diagnostic	1:100
H_4R	Rabbit Anti-Mouse (Rat) IgG	Santa Cruz Biotechnology	1:250
secondary antibody	Anti Rabbit IgG	Sigma-Aldrich	1:12500

Table 4.

Specifications of antibodies used in Western blot studies

The table summarizes the isotype, source, dilution and commercial availability of the antibodies applied in western blot measurements.

13. Statistics

Statistical analysis was performed using analysis of variance (ANOVA), as appropriate, and Tukey test as post hoc test. Statistical significance (p value) less than 0.05 was considered as statistically significant ($p<0.05$ is indicated by *, $p<0.01$ is indicated by ** and $p<0.001$ is indicated by ***). The Statistica program version 8 was used for statistical analysis except adhesion studies where Origin 7.0 software was applied.

Human studies

1. Monocyte separation and *in vitro* differentiation of DCs

Buffy coats were obtained with the written permission of the National Blood Transfusion Centre, Budapest Hungary. Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll gradient centrifugation (Amersham Biosciences, Uppsala, Sweden). $CD14^+$ monocytes were isolated from PBMC by immunomagnetic cell separation using anti-CD14-conjugated microbeads (Miltenyi Biotech). To obtain IDCs $CD14^{high}$ monocytes (>97%) were cultured in 6-well plates at a density of 2×10^6 cells/ml in serum-free AIMV medium (Life technologies, Carlsbad, California) supplemented with antibiotics, 100 ng/ml IL-4 (PeproTech Inc., Rocky Hill, NJ, USA) and 80 ng/ml GM-CSF (PeproTech Inc.). At day 2, the same amount of cytokines was added and the cells were cultured for another 3 days. Resting DCs were harvested on day 5, or were activated by LPS (100 ng/ml) and IFNγ (10 ng/ml, PeproTech Inc.) and harvested on day 6. When indicated, histamine (10 µM) or histamine combined with

specific HR inhibitors (10 µM) were added at day 0 and day 2 together with the differentiating cytokines and when activated with LPS+IFNγ (100 ng/ml and 10 ng/ml respectively). The following HR antagonists were used: pyrilamine (H_1R antagonist, Sigma-Aldrich), famotidine (H_2R antagonist), JNJ7777120 and JNJ10191584 (H_4R antagonists).

2. Phenotypic characterization of DCs

Phenotyping of resting and activated DCs was performed by flow cytometry using anti-CD83-PE (R-Phycoerythrin, R&D Systems Inc.) and anti-CD1a-APC (Allophycocyanin, Biolegend, San Diego, CA, USA) antibodies (Beckman Coulter, Hialeah, FL, USA) and isotype-matched control Ab (BD Pharmingen, San Diego, CA, USA). For measuring H_2R expression by DC subsets the washed cells were labelled with 0.1µg rabbit anti-human H_2R (Alpha Diagnostic International Inc., San Antonio, TX, USA) in the presence of heat-inactivated normal mouse serum as a blocking reagent. After 30 min incubation on ice the cells were washed and labeled with 1µg (0.5µl) Alexa488-conjugated goat anti-rabbit $(Fab')_2$ fragments (Invitrogene, Eugene, OR, USA) together with 5ul PE-labelled mouse anti-human CD1a antibody (BD Biosciences). Fluorescence intensities were measured by FACS Calibur (BD Biosciences, Franklin Lakes, NJ), data were analyzed by the FlowJo software (Tree Star, Ashland, OR, USA). The $CD1a^-$ and $CD1a^+$ cells were separated with FACS DiVa high-speed cell sorter (BD Biosciences).
A 633 nm red diode laser excitation was used for APC-conjugated CD1a staining, and a 488 nm blue Argon laser excitation was used for PE-conjugated CD83 staining. R-PE emission was measured in the FL2 channel of the instrument with 585/42 nm band pass (BP) filter (reflected first by 560 nm short pass (SP) dicroic mirror (DM) then 640 nm long pass (LP) DM). APC emission was measured in the FL4 channel of the instrument with 661/16 nm BP (reflected first by 560 nm SP DM then passed through the 640 nm LP DM). The CD83 border on the CD83-CD1a plots was set with the help of the isotype-matched control IgG1-PE+CD1a-APC double staining of the same samples. As the $CD1a^{low/-}$ and $CD1a^{high/+}$ monocyte-derived DCs were well distinguishable by eye in the flow cytometric plots, we have drawn the CD1a border on the individual plots by eye instead arbitrarily and avoided the IgG1-APC control staining.

3. Real-time quantitative reverse transcriptase-polymerase chain reaction (QRT-PCR)

Total RNA was isolated from DCs by Trizol reagent (Invitrogen). Reverse transcription was performed at 37 ^oC for 120 min from 100 ng total RNA using the High Capacity cDNA Archive Kit (Applied Biosystems). QRT-PCR for H_1R, H_2R, H_3R H_4R, MMP-9 and MMP-12

genes was performed by ABI PRISM 7900 (Applied Biosystems) with 40 cycles at 94 ^{o}C for 12 sec and 60 ^{o}C for 60 sec using Taqman gene expression assays (Applied Biosystems). All PCR reactions were run in triplicates with a control reaction containing no RT enzyme. The comparative Ct method was used to quantify transcripts relative to the endogenous control genes GAPDH or 36B4.

Gene symbol	*Assay code*
MMP-9	Hs00234579_m1
MMP-12	Hs00899662_m1
36B4	Hs00420895_gH
GAPDH	Hs02758991_gH

Table 5.

Quantitative real-time PCR primers and probes

The table summarizes the specifications of the primers applied in quantitative real-time PCR measurements.

4. Cytokine measurements

Culture supernatants of LPS+IFNγ-activated DCs were harvested 24 hours after LPS treatment and the concentration of IL-6 and IL-10 was measured by using OptEIA kits (BD Biosciences).

5. Migration assay

DCs were suspended at 10^6 cells/ml migration medium (0.5 % BSA in RPMI 1640). Transmigration inserts (with 6.5 mm diameter and 5 µm pore size) were obtained from BD Biosciences. The MIP-3β chemokine (PeproTech Inc.) was diluted at 200 ng/ml in migration medium and added to the lower chambers in a final volume of 600 µl. DCs were added to the upper chamber in a final volume of 250 µl and the chemotaxis assay was conducted for 4 hours in 5% CO_2 at 37 ^{o}C. At the end of the assay, the inserts were discarded and cells migrated to the lower chamber were collected. The number of cells migrated was counted by using polystyrene standard beads (Sigma-Aldrich) by flow cytometry.

6. Chemokine array

Chemokine gene expression profiling of $CD1a^-$ and $CD1a^+$ DCs was studied with the Human Chemokines & Receptors PCR Array assay (SABiosciences^{TM}, Frederick, MD, USA) in accordance with the manufacturer's recommendations. Briefly, total RNA was isolated from the different cell populations using RNeasy Mini Kit. After DNase I digestion, first strand

cDNA synthesis was performed by using the RT^2 First Strand Kit (SABiosciences™) following the manufacturer's instructions and including a genomic DNA elimination step and external RNA controls. For each sample, 900 ng of total RNA was reverse transcribed. Real-time PCR measurement was performed using the RT^2 qPCR Master mix (SABiosciences™) according to the manufacturer's instructions on the ABI Prism 7000 real-time PCR platform. For each analysis, 25 µl of the experimental cocktail of the cDNA samples and RT^2 qPCR Master mix was distributed across the PCR array 96 well plates, each of which contained 84 wells with a real-time PCR assay for different chemokine genes, 5 wells with assays for different housekeeping genes, a genomic DNA control, 3 replicate Reverse Transcription controls, and 3 replicate Positive PCR controls. A two-step cycling program was used consisting of an initial activating step of 10 min at 95 ºC and a second step of 40 cycles of denaturation (95 ºC, 15 s) and annealing (60 ºC, 1 min). Finally, each plate underwent a melting curve program (95 ºC, 1 min; 65 ºC, 2 min (optics off); 65 ºC to 95 ºC at 2 ºC/min (optics on). Gene expression levels were determined as the inverse of the Ct value. Ct values greater than 35, or those not detected, were set to 35. After cycling with real-time PCR, the amplification data were analyzed and statistical significance was calculated by SABiosciences on-line software.

6. RESULTS

Mouse studies

1. HR expression of mouse splenic DCs

The presence of different HRs can be monitored directly at the mRNA and protein levels, or indirectly by testing the effects of various receptor-specific ligands. Concerning HR expression on DCs the available data are often contradictory, especially those of H_3R [60] [61]. Thus we first investigated HR expression at the protein level in splenic $CD11c^+$ DCs by Western blot and flow cytometry. As H_3R expression could not be detected by real-time PCR this receptor was not tested further. H_1R was detected at 55 kDa, H_2R at 40 kDa, while H_4R showed a 44 kDa band by Western blot [79] [80] (Figure 8.). Beside the specific protein band of H_4R another band of larger-size was also detected on the membrane, which could correspond to a glycosylated form of the receptor. Flow cytometric analysis also confirmed the cell surface expression of H_1R, H_2R and H_4R on splenic DCs (Figure 9.). Based on these results we concluded that the H_1R, H_2R and H_4R were expressed in mouse splenic DCs.

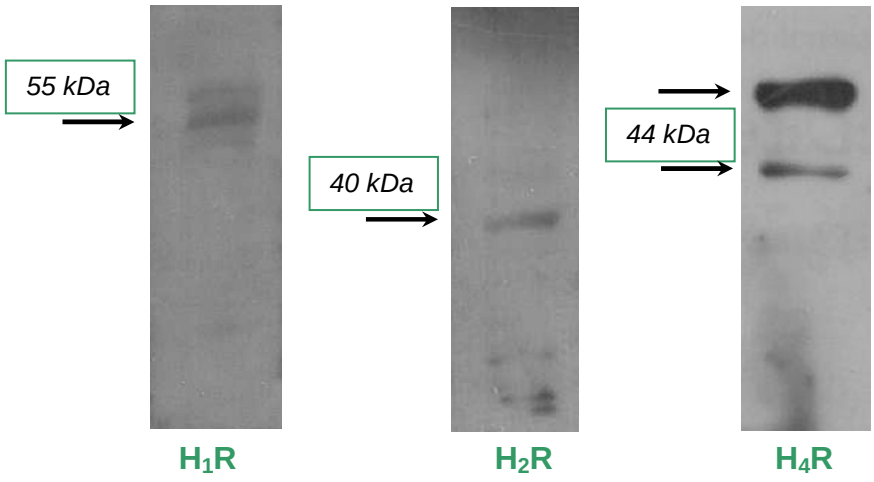


Figure 8.

H_1R, H_2R and H_4R expression in spleen-derived DCs (Western blot)

The expression of HRs in DCs was determined by immunoblot analysis. After cell lysis the protein bands were separated by electrophoresis, transferred to membranes and were probed with receptor-specific primary antibodies (see Table 4.). The appropriate HRP-conjugated secondary antibodies were used to visualize protein bands corresponding to H_1 H_2 and H_4 receptors. Numbers indicate the molecular sizes of the proteins: H_1R: 55 kDa, H_2R, 40 kDa, H_4R: 44 kDa, respectively. A typical result from one of three independent experiments is shown.

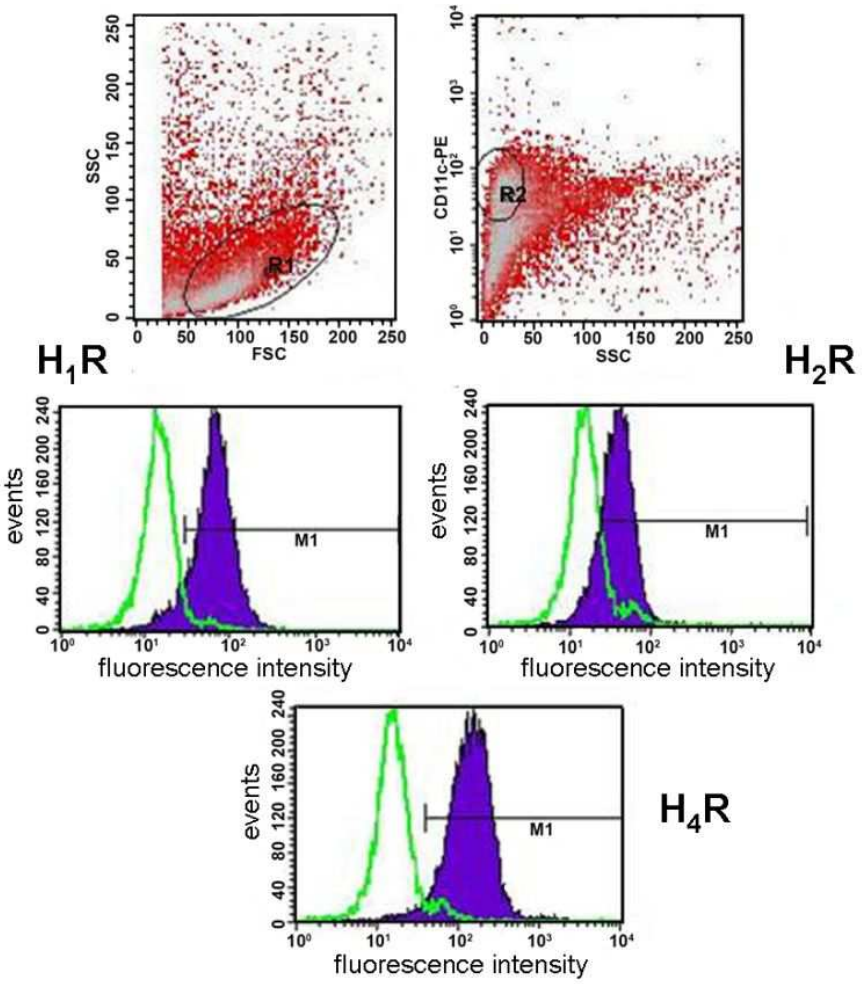


Figure 9.

Expression of HRs on splenic DCs (flow cytometry)

Detection of HR expression (H_1R, H_2R and H_4R) was assessed by flow cytometry after permeabilization of spleen-derived DCs using a multiple step staining protocol. Based on the forward and side scatter (FSC-SSC) dot plots DCs were identified by CD11c cell surface expression within the morphologically viable cell population. Histograms showing $CD11c^+$ morphologically viable cells demonstrate H_1R, H_2R and H_4R expressions. Green lines represent staining with anti-rabbit IgG antibody served as control. M1 marker indicates the difference between the control and specific HR staining intensities. Data of a representative experiment out of three is shown.

2. Changes in HR expression during DC differentiation and activation

We monitored HR expression of bone marrow-derived $CD11c^+$ DCs on days 6, 8, 10 and 13 of *in vitro* differentiation by flow cytometry. H_1R expression did not change remarkably, while H_2R (Geometric mean on day 6: 340 ± 81; on day 13: 194 ± 26) and H_4R (Geometric mean on day 6: 547 ±; 28; day 13: 338 ± 36) expression was significantly reduced during the course of DC development. When DCs were treated with LPS on day 13 and tested

for HR expression 24 hours later by flow cytometry we observed that the expression of H_1R and H_2R did not alter, while the surface expression of H_4R elevated significantly as a result of LPS activation (Geometric mean on day 13: 338 ± 36; after LPS treatment: 479 ± 106) (Figure 10.).

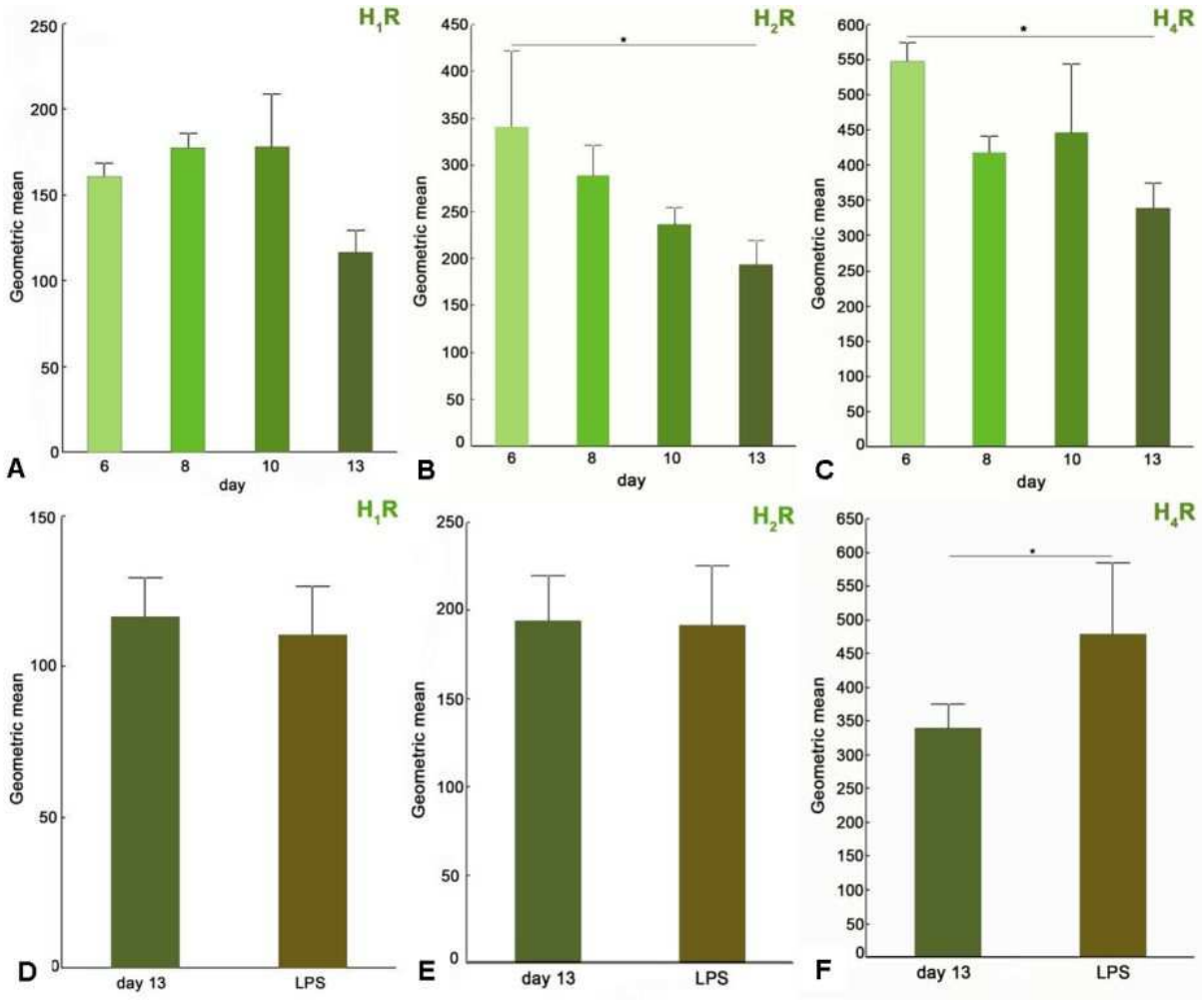


Figure 10.

Changes in HR expression in the course of *in vitro* differentiation and activation

Detection of H_1R (A), H_2R (B) and H_4R (C) expression on different days of DC differentiation from bone marrow (day 6, 8, 10 and 13; flow cytometry).On day 13 DCs were treated by LPS, for 24 hours and after staining the measurement of HRs was performed by flow cytometry (D, E, F). Values shown are means ± SEM of 4 independent experiments. Statistical significance was calculated using one-way ANOVA, and Tukey as post hoc test. (: $p<0.05$).*

3. In vitro effect of histamine on H_4R and the peptide-presenting function of DCs

DCs are potent antigen presenting cells and the proper presentation of altered self- and/or foreign antigens to T lymphocytes is essential to maintain cell tolerance and/or antigen-

specific T-cell activation and effector cell functions. Using an *in vitro* assay we first tested the effect of histamine on the peptide-presenting capacity of splenic $CD11c^+$ DCs. DCs separated from the spleens of WT mice were co-cultured with the aggrecan-specific 5/4E8 T cell hybridoma cells in the presence or absence of human aggrecan peptide for 24 hours. Histamine was added to the co-cultures at different concentrations (0.1, 1, 10, 100 µM). As a result of peptide-specific activation, the hybridoma cells produced IL-2 cytokine in the range of 4000-10000 pg/ml, while in the absence of antigen T cell activation did not occur. To verify that the activation of IL-2 production by histamine is not a direct effect on T cells (without the participation of DCs) control wells containing only 5/4E8 cells, antigen or different concentrations of histamine were also set up where IL-2 levels remained undetectable. These results suggest that histamine can significantly reduce IL-2 secretion of T cells *in vitro* (Control: 100% ± 6; 1 µM histamine: 80% ± 5) (Figure 11.A, $p<0.05$).

The role of H_2R and H_4R in some DC functions has previously been shown [61]. As a next step we investigated whether H_4R played a role in the histamine-mediated effect on antigen presentation. DCs were treated with different concentrations of the H_4R agonist 4-MH (1, 5, 10, 50, 100 µM), and the level of IL-2 was detected. Among the concentrations tested, 100 µM 4-MH could significantly reduce the antigen presenting capacity of DCs (Control: 100% ± 3; 100 µM 4-MH: 72 ± 5) (Figure 11.B, $p<0.05$). The role of histamine was further confirmed by using the specific receptor blockers; famotidine for H_2R and JNJ10191584 for H_4R both added at 10 µM 1 hour before histamine treatment. Antagonist-treated wells served as controls. We found that the H_2R antagonist further reduced the antigen presenting capacity of DCs, while the H_4R blocker restored the histamine-mediated reduced antigen presentation (histamine: 73% ± 3; histamine+JNJ10191584: 79% ± 1) (Figure 12.A, $p=0.057$). These results indicate that the H_4R specifically affects the histamine-mediated reducing effect on DC peptide presentation.

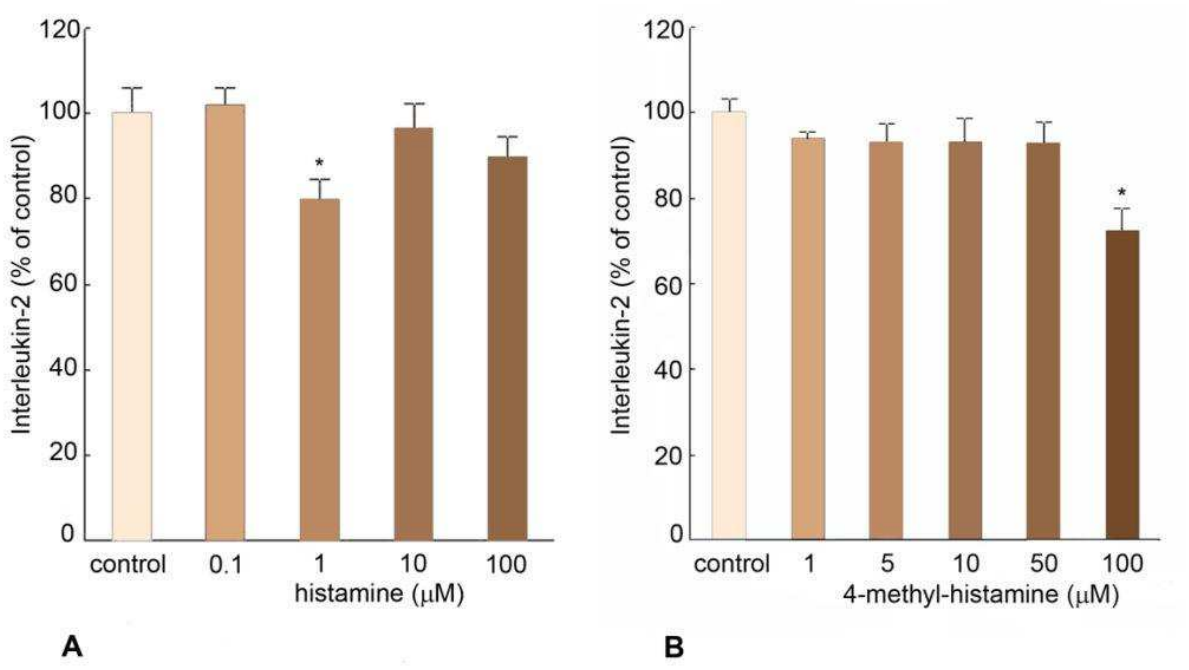


Figure 11.

Effect of histamine and 4-MH on *in vitro* antigen presentation of spleen-derived DCs

WT splenic DCs treated with different concentrations of histamine (A; n=8) or 4-MH (B; n=3) were cultivated with 5/4E8 T cells and human aggrecan peptide, and the IL-2 production representing the antigen-specific T cell response was determined by ELISA. Values shown are given as means ± SEM percentage of control. Statistical significance was calculated by one-way ANOVA and Tukey HSD, as post hoc test (: p<0.05).*

In the next experiment peptide presentation of WT and $H_4R^{-/-}$ DCs was compared. As shown in Figure 12.B DCs that do not express functional H_4R on their surface possess significantly higher antigen presenting capacity (182% ± 8) than WT cells (100% ± 6) (Figure 12.B, $p<0.001$).

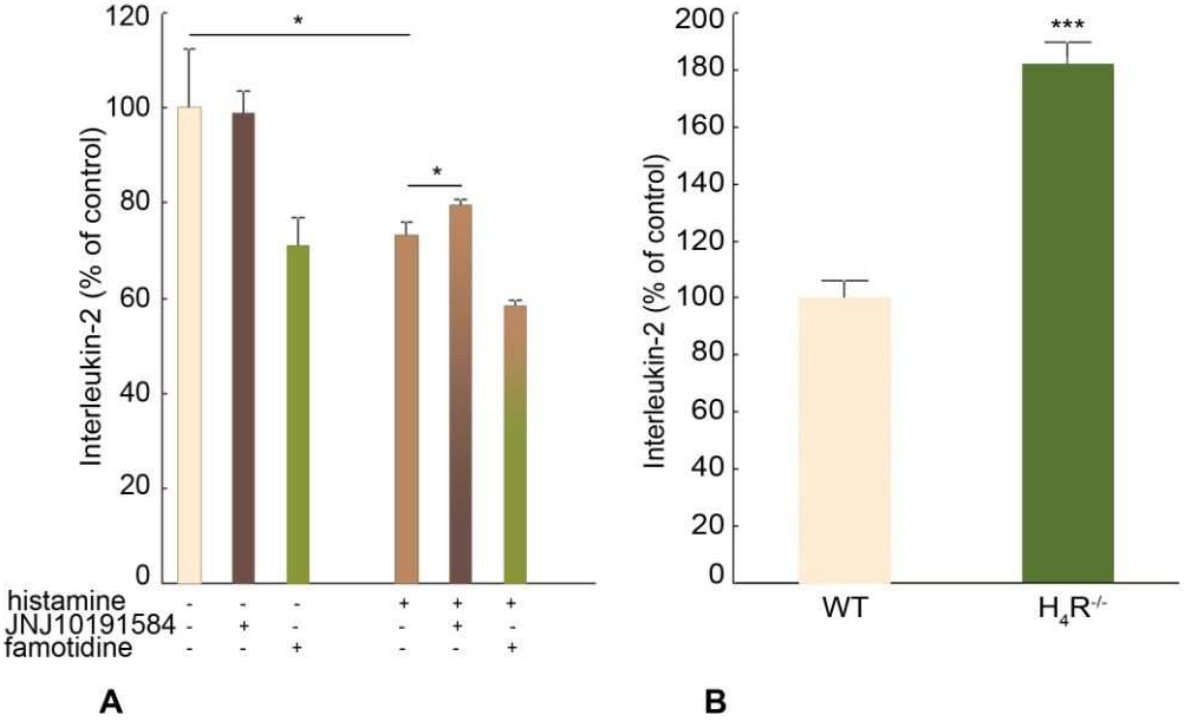


Figure 12.

Role of H_4R in DC antigen presentation

Effect of 1 hour H_2R (famotidine, 10 µM) and H_4R (JNJ10191584, 10 µM) antagonist treatment on the antigen presentation of histamine-treated (1 µM) WT DCs (A; n=8; main effects ANOVA). Comparison of WT and $H_4R^{-/-}$ DCs antigen presentation (B; n=4; one way ANOVA). Values shown are given as means ± SEM percentage of control. Statistical significance was calculated by ANOVA and Tukey HSD, as post hoc test (: p<0.05, ***: p<0.001).*

4. Comparison of the expression of costimulatory molecules in WT and $H_4R^{-/-}$ DCs

Several data revealed that the pattern of surface molecules on the DCs' membrane has an important role in T cell activation. Thus, we investigated whether WT and $H_4R^{-/-}$ DCs differed in the cell surface expression of characteristic DC proteins and costimulatory molecules and thus could be responsible for altered peptide presentation and T cell-derived IL-2 production.

First WT and $H_4R^{-/-}$ histamine-treated (1 µM, 4 and 19 hours) or untreated splenic DCs were studied by flow cytometry. Neither the expression of the DC marker CD11c, nor MHCII or the costimulatory molecules CD40, CD80, CD86 was altered significantly in the presence of histamine when compared in the two animal groups (Figure 13.).

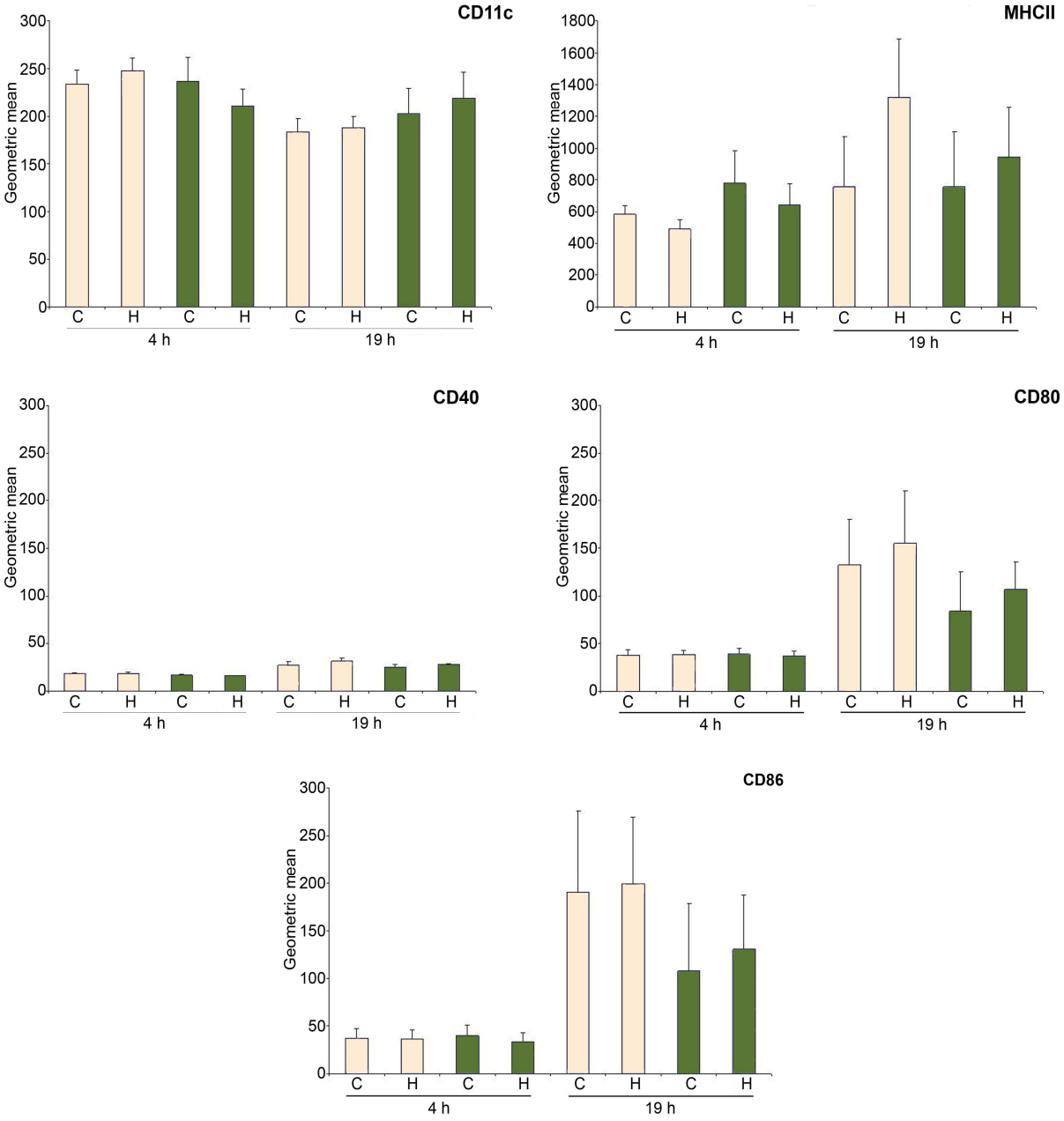


Figure 13.

Expression of characteristic surface molecules on untreated (C) and histamine-treated (H) spleen-derived WT (■) and $H_4R^{-/-}$ (■) DCs

Results of flow cytometric analysis of cell surface markers and costimulatory molecules on splenic WT and $H_4R^{-/-}$ DCs after 4 and 19 hours histamine treatment (1 µM). Values shown are means ± SEM of 4 independent experiments. Statistical significance was calculated using two-way ANOVA and Tukey as post hoc test.

Next, *in vitro* generated DCs were applied, to which different concentrations of histamine (1 µM, 10 µM) were added on day 13 of differentiation, then 24 hours later the

characteristic surface- and costimulatory molecules were measured by flow cytometry. Surface expression of the molecules tested did not differ significantly between WT and $H_4R^{-/-}$ DCs or after histamine treatment (Figure 14.). Based on these results the differences found in the antigen presenting capacity of DCs could not be attributed to changes in cell surface molecule expression of the cells.

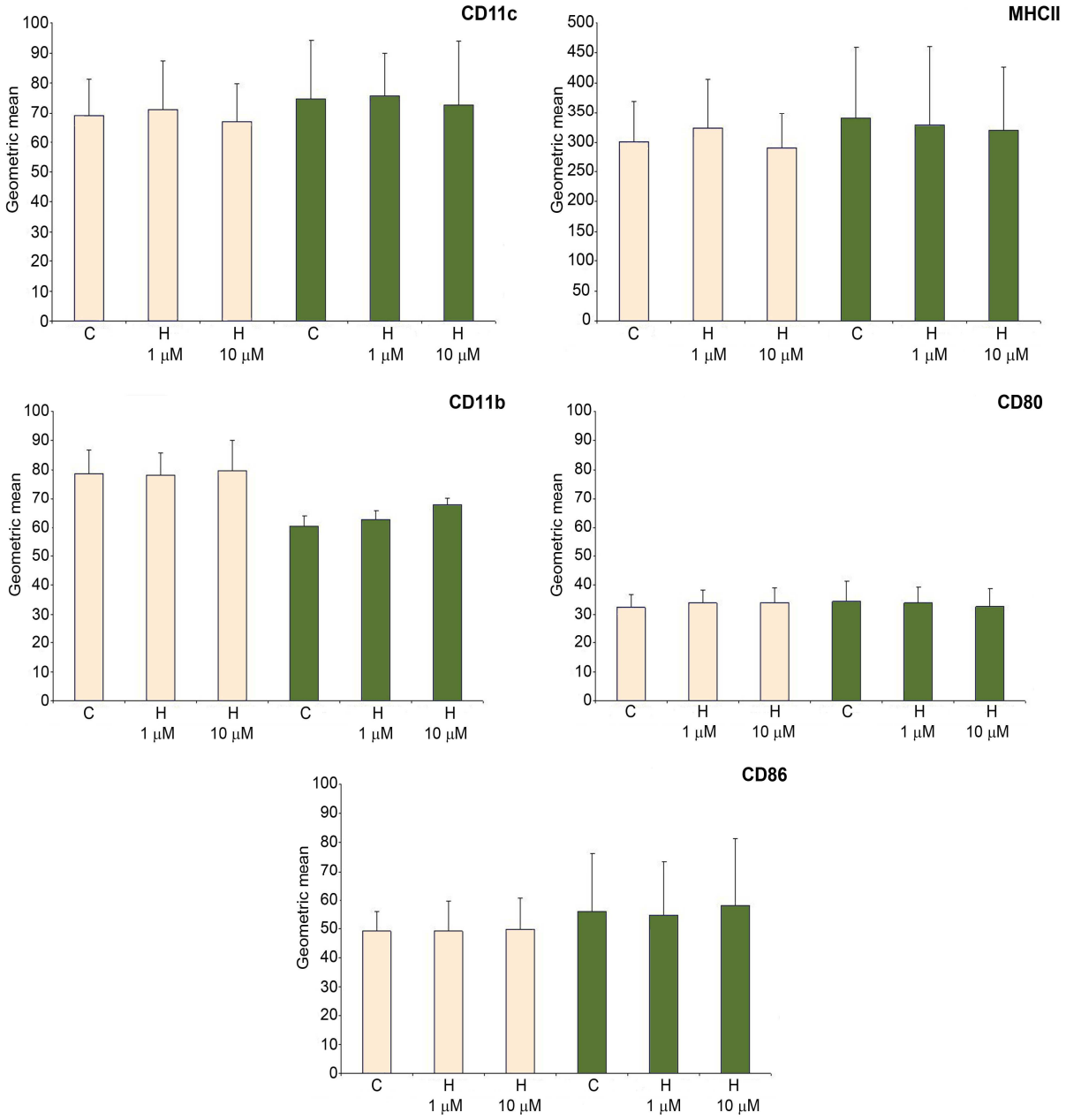


Figure 14.

Expression of surface markers of differentiated untreated (C) and histamine-treated (H) WT (■) and $H_4R^{-/-}$ (■) DCs

On day 13 of in vitro differentiation WT and $H_4R^{-/-}$ DCs were treated with histamine (1 µM, 10 µM), and after 24 hours the expression of characteristic surface- and costimulatory molecules was detected by flow cytometry. Values shown are means ± SEM of 4 independent experiments. Statistical significance was calculated using two-way ANOVA and Tukey as post hoc test.

5. Role of histamine and H_4R in in vitro migration of DCs

The sentinel function of DCs requires continuous patrolling of blood, peripheral tissues, lymphatic vessels and lymphoid organs supported by constant migration. Histamine and H_4R was shown to have a role in eosinophil, mast cell and human DC migration and chemotaxis [47].

The role of histamine and H_4R in the migration of splenic- or bone marrow-derived DCs was investigated with the help of an *in vitro* migration assay. First the effect of different concentrations of histamine (1, 10, 100 µM) or 4-MH (1, 10, 50 µM) was tested on splenic DC chemotaxis in Transwell system. The number of transmigrated cells was detected by flow cytometry normalized to a defined number of microparticles. Neither different concentrations of histamine, nor the H_4R agonist 4-MH could influence DC migration significantly (Figure 15.A and B).

Then we hypothesized that prolonged histamine treatment, if applied during DC differentiation may induce changes in the migratory capacity of the cells. To investigate this possibility DCs were differentiated in the presence or absence of 1 µM histamine added on days 0 and 3; 6 and 8 or 0, 3, 6 and 8 of *in vitro* cultures. On day 10, the migration of DCs was studied in Transwell system. Figure 15.C shows a trend of stimulated DC migration by histamine in a time-dependent manner, but this increase was not statistically significant.

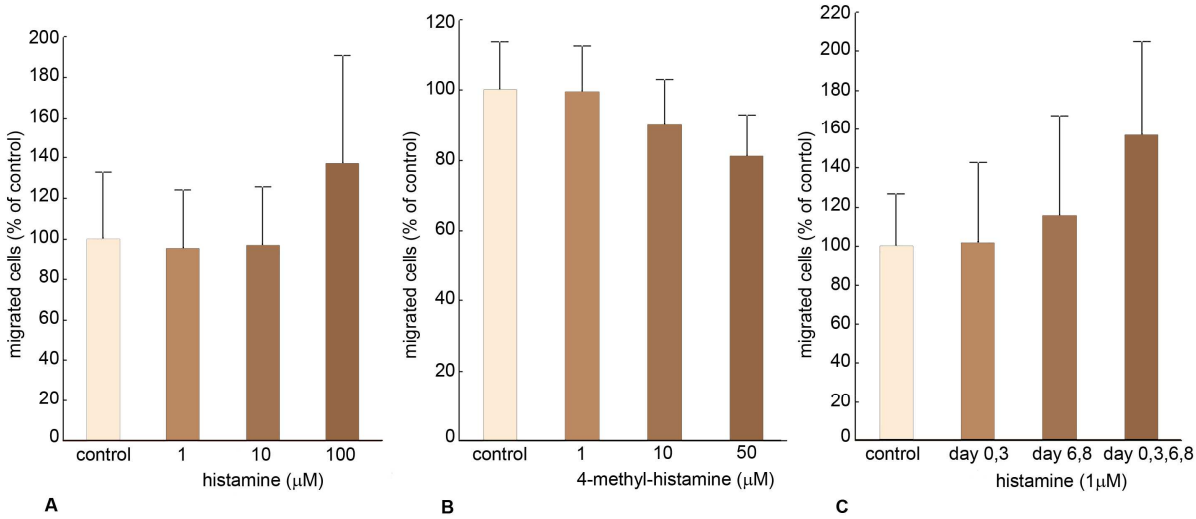


Figure 15.

Effect of histamine and 4-MH on the migration capacity of WT DCs

Results of migration studies performed in in vitro Transwell system after treatment with different concentrations of histamine (A; n=6; one-way ANOVA) and 4-MH (B; n=12; one-way ANOVA). Migration of bone marrow-derived DCs treated with histamine during differentiation (C; n=5; one-way ANOVA). Values shown are means ± SEM of independent experiments. Statistical significance was calculated using ANOVA and Tukey as post hoc test.

Finally, migration of WT and $H_4R^{-/-}$ DCs tested on day 10 of bone marrow-derived DC differentiation was also compared in Transwell chambers. Results shown in Figure 16. indicate that DCs derived from H_4R-deficient mice show significantly lower migratory capacity (52145 ± 6531) than their WT counterparts (73303 ± 2883).

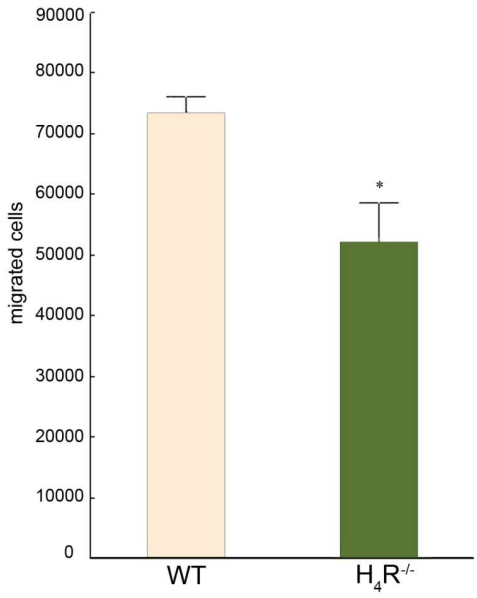


Figure 16.

Comparison of the migration capacity of WT and $H_4R^{-/-}$ DCs

Migration capacity of bone marrow-derived differentiated DCs of WT and $H_4R^{-/-}$ mice. The number of DCs migrating through the Transwell membrane, was measured by flow cytometry and were normalized to $2x10^4$ microparticles. Values shown are means ± SEM of 7 independent experiments. Statistical analysis was performed using one-way ANOVA and Tukey as post hoc test. (: $p<0.05$)*

6. Adhesion studies

Migration is a complex process that can be influenced by cytokines, chemokines and adhesion molecules. To ascertain the functional background of the difference between WT and $H_4R^{-/-}$ DC migration adhesion studies were performed. Differentiated DCs were plated onto 96 well plates supplied with special electrodes suitable for measuring cell adhesion.

Time course characteristics of DC adhesion are shown in Figure 7. (see Methods section). After plating, the cells attach and spread relatively fast, which is detected as a steep increase of Cell Index (CI) values. The adhesion curve of DCs treated with 1 µM histamine shows similar slope with the control, albeit with a faster kinetic (Figure 17.A). 2 hours after seeding the cells addition of histamine induces a significant increase in CI value as compared to the control (CI control: 1.20 ± 0.01 ; histamine: 1.28 ± 0.03) (Figure 17.B).

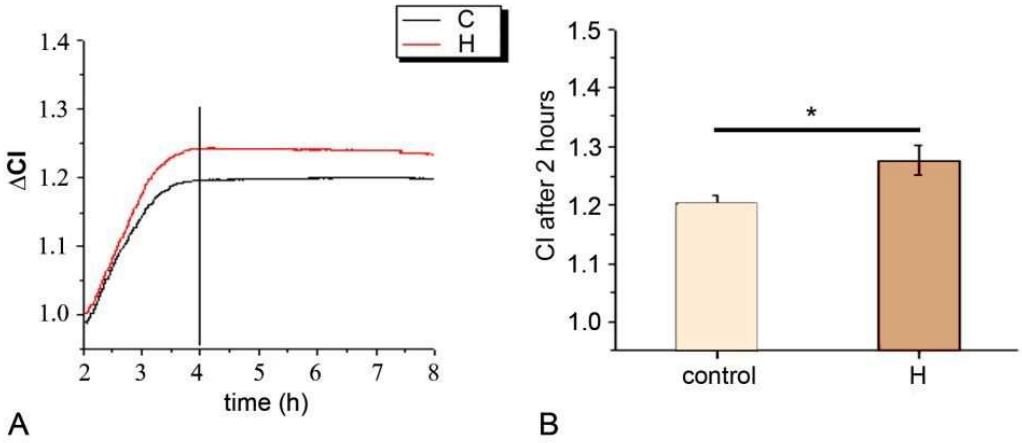


Figure 17.

Effect of an acute histamine treatment on DC adhesion

Adhesive capacity of DCs was measured in a real-time system. Calculation of ΔCI was performed for the baseline. Detection of time course characteristics of the adhesion curve (A), and CIs of control and 1 µM histamine-treated (H) cells after 2 hours (B) are shown. Values shown are means ± SEM of 5 independent experiments. Statistical significance was calculated using one-way ANOVA (: p<0.05).*

In the next experiment we investigated if a chronic histamine challenge during DC differentiation may influence adhesion of the cells. Cells were treated with different concentrations of histamine (1 µM and 10 µM) on day 0, 3, 6 and 8 of differentiation and cell adhesion was measured on day 10. Histamine in both concentrations could enhance CI values compared to the control (CI: 1.19 ± 0.01), however pretreatment with 1 µM histamine (CI: 1.30 ± 0.02) was more effective in increasing cell adhesion than the 10 µM concentration (CI: 1.24 ± 0.02) (Figure 18.). Comparing the slopes of the adhesion curves demonstrated that 1 µM histamine applied during DC differentiation generated a faster and on the whole an

increased adhesion, which could not be influenced by additional histamine applied right before the experiment (Figure 19.).

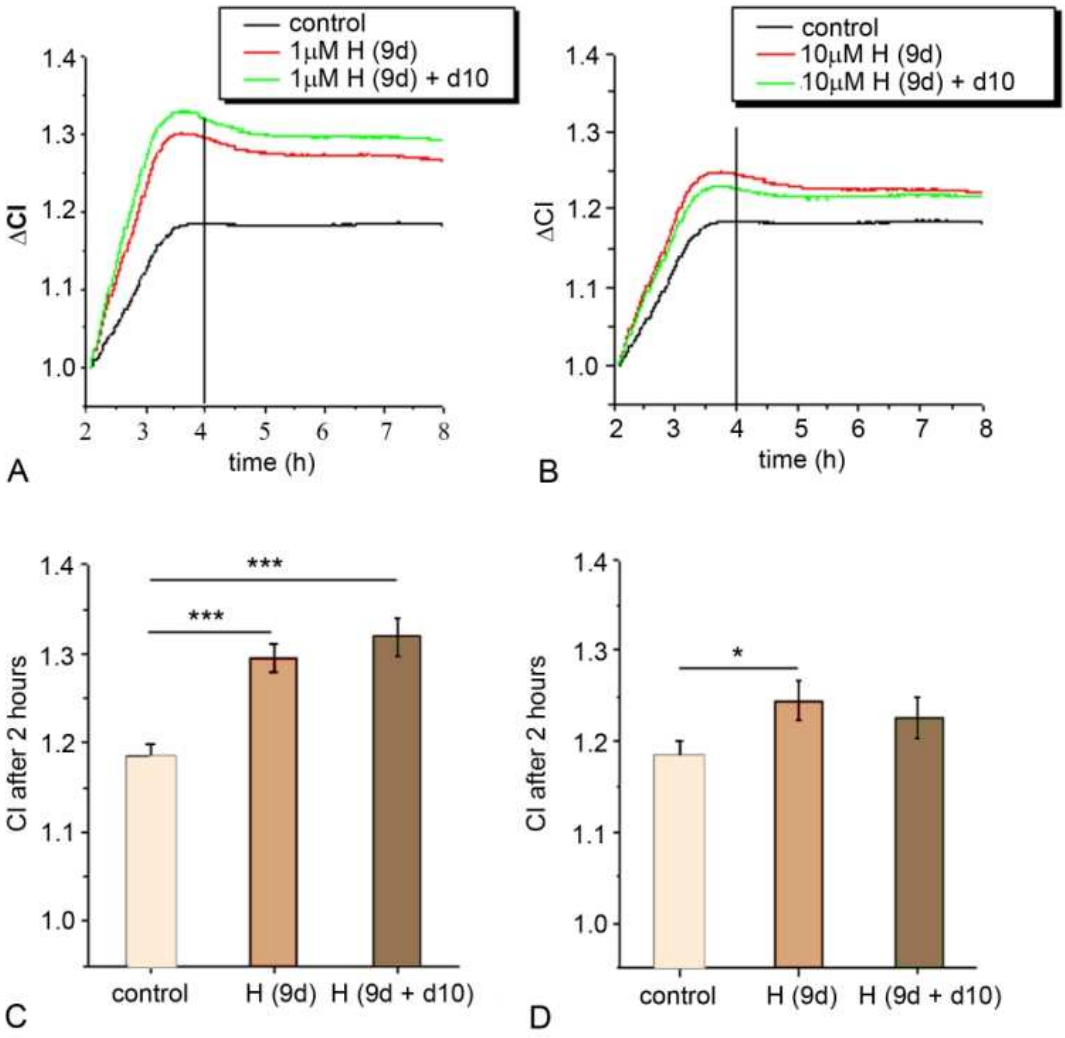


Figure 18.

Effect of a chronic histamine treatment on DC adhesion

Monitoring the effect of 1 μM and 10 μM histamine added during in vitro differentiation on the adhesion characteristics of DCs. Detection of time course characteristics of the adhesion curve (A, B), and comparison of CIs of control, histamine treated (H) (during differentiation (9 days)) and histamine pretreated (during differentiation and on the day of measurement (9days+day 10)) DCs after 2 hours (C, D). Values shown are means ± SEM of 3 independent experiments. Statistical significance was calculated using one-way ANOVA (: p<0.05, **: p<0.01, ***: p<0.001).*

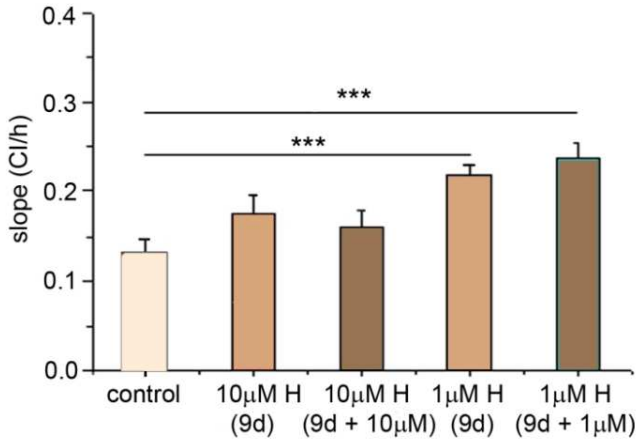


Figure 19.

Effect of a chronic histamine treatment on DC adhesion

*Monitoring the effect of 1 μM and 10 μM histamine (H) added during in vitro DC differentiation on the adhesion characteristics of the cells. Calculation of the slopes was done after 1.5 hours of cell plating. Values shown are means ± SEM of 3 independent experiments. Statistical significance was calculated using one-way ANOVA (***: p<0.001).*

When we compared the adhesive properties of WT and $H_4R^{-/-}$ DCs, cells of different genotypes had similar adhesion characteristics, but in the first period (72 min) the average CI of $H_4R^{-/-}$ cells showed a faster increase than that of the WT cells. Five hours after seeding the difference of the two cell types was diminished. The fast phase (72 min) of cell adhesion is also characterized by the slopes of the curves, and showed significant difference of the two investigated DCs; the $H_4R^{-/-}$ curve had increased CI value (0.23 ± 0.03) (Figure 20.A). About 120 min after seeding, the CI showed significantly different values in WT and $H_4R^{-/-}$ cells (0.19± 0.01 and 0.26± 0.02), respectively (Figure 20.B).

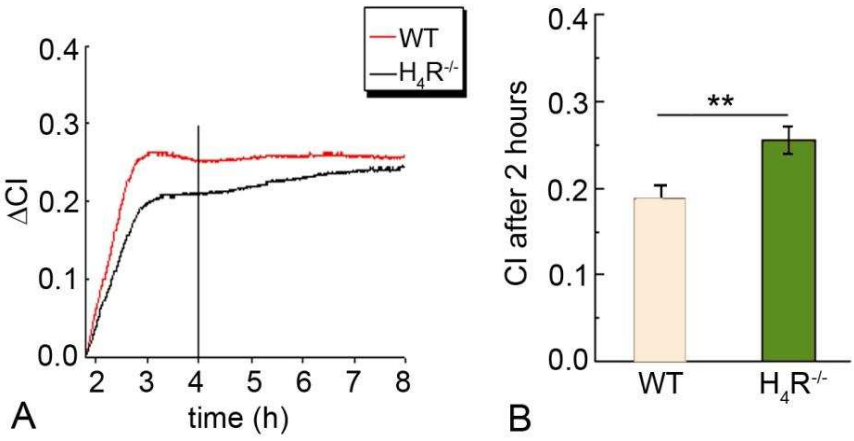


Figure 20.

Adhesive properties of WT and $H_4R^{-/-}$ DCs

*Attachment and spreading of WT and $H_4R^{-/-}$ DCs on microelectrode sensors were monitored by a real-time cell electronic sensing system. The ΔCI was calculated for the baseline at the time of the cell seeding and represent the average value of 6 parallel experiments in triplicate. (A) Average of the slope values of the CI curve for the first 72 minutes (n = 6). (B) Average ΔCI data at 2 hours after seeding (n = 6, **: p<0.01).*

To test whether H_4R is the HR responsible of the difference shown before, WT DCs were treated with H_1R, H_2R and H_4R antagonists (10 µM) in the presence or absence of histamine and cell adhesion was measured. Blocking of H_1R and H_2R did not result in a considerable change as compared to control or histamine treated cells, while the H_4R antagonist JNJ10191584 could effectively reduce (H+JNJ10191584 CI: 1.22 ± 0.01) the increasing effect of histamine on DC adhesion (H CI: 1.28 ± 0.03) (Figure 21.).

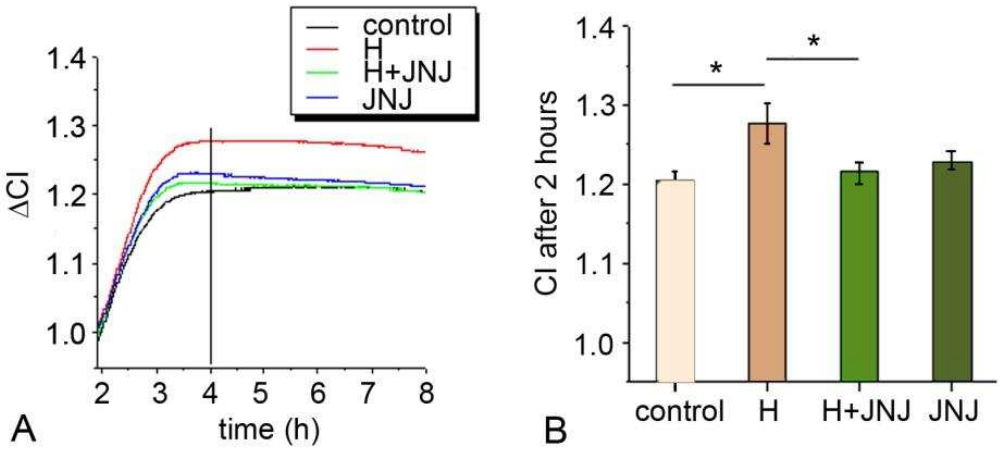


Figure 21.

Role of H_4R in DC adhesion

Monitoring the effect of histamine (H), H_4R antagonist JNJ10191584 or histamine in combination with H_4R antagonist applied on day 10 of in vitro differentiation on the adhesion of DCs. Detection of time course characteristics of the adhesion curve (A), and comparison of CIs after 2 hours (B). Values shown are means (± SEM) of 5 parallel experiments. Statistical significance was calculated using one-way ANOVA (: p<0.05).*

7. Effect of H_4R on cytokine production of in vitro stimulated DCs

Functional characteristics of DCs can effectively influence the type of antigen-specific immune response by polarizing T cells toward Th1, Th2, Th17 or Treg directions. This subtly-regulated process is not only influenced by the interacting cells but also by chemical mediators, cytokines and chemokines acting through their specific cell surface receptors. In our *in vitro* system we investigated the cytokine response of activated splenic DCs after H_4R stimulation. Separated splenic DCs were pretreated with the H_4R agonist 4-MH (0.1 µM) for 15 min followed by LPS activation for 24 hours. In order to clarify the role of H_4R, we used the specific antagonist JNJ7777120 (10 µM), added to cells 15 min before agonist challenge.

Real-time PCR measurements revealed that the mRNA expression of IL-1ß, one of the paramount inflammatory cytokines, was elevated after a combined treatment with a H_4R agonist and LPS and this significant effect could be reversed by the H_4R blocker JNJ7777120 (Figure 22.). Importantly, no significant alteration was observed in the expression of the IL-6, IFNγ, IL-12a, IL-10 cytokines and the CCR7, CCL2, CCL4, CCL1, chemokines after H_4R stimulation.

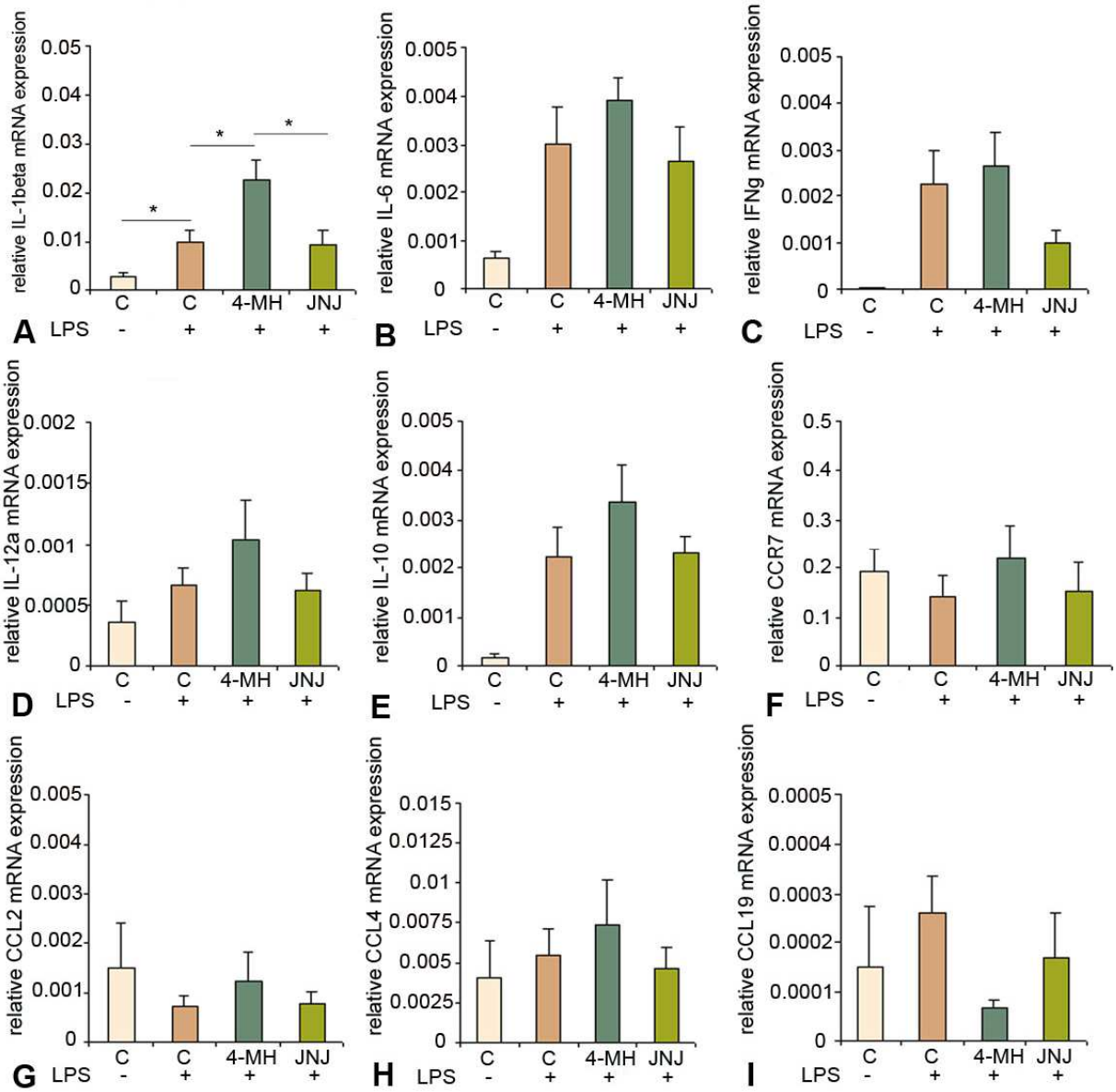


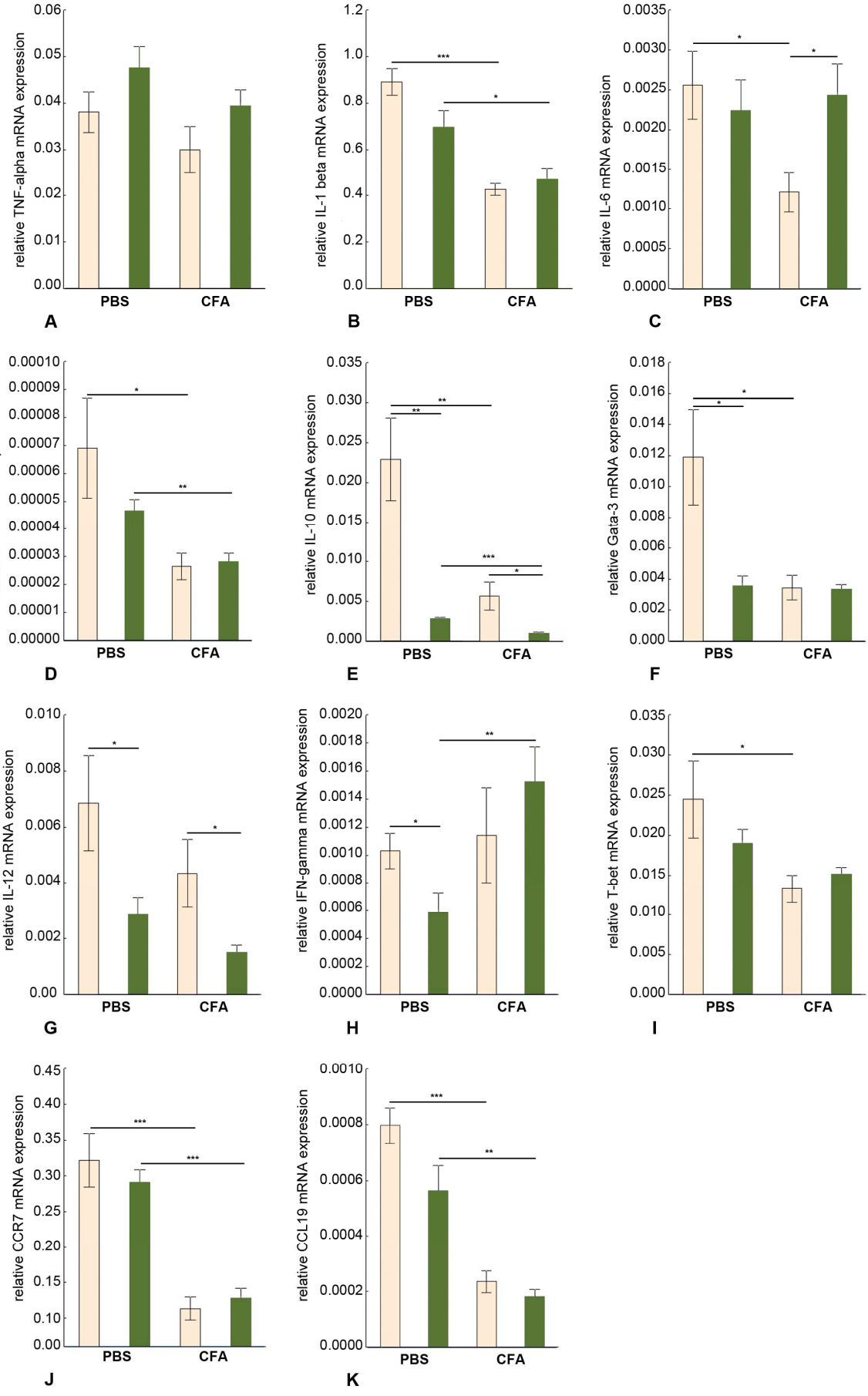
Figure 22.

Effect of *in vitro* H_4R stimulation on the cytokine response of WT spleen-derived DCs

Splenic DCs were treated with a H_4R agonist, 4-MH (0.1 µM) for 15 min alone or along with a H_4R antagonist, JNJ7777120 (10 µM) for 15 min prior to agonist challenge, followed by LPS (1 µg/ml) treatment. After 24 hours the expression level of different cytokines and chemokines were measured by real-time PCR. Values shown are means ± SEM of 4 independent experiments. Statistical significance was calculated using one-way ANOVA and Tukey as post hoc test. (: $p<0.05$).*

8. Cytokine responses of in vivo activated WT and $H_4R^{-/-}$ DCs

Most data on DC cytokine production are based on the results of *in vitro* experiments. To obtain more information about DC cytokine expression, we carried out an *in vivo* assay and compared the cytokine profiles of WT and $H_4R^{-/-}$ DCs. WT and $H_4R^{-/-}$ mice were injected intraperitoneally with CFA. On day 7 after injection, we monitored the expression of various cytokines, chemokines and major transcription factors involved in Th1, Th2 responses and Treg functions of isolated splenic DCs by real-time PCR. We detected significantly lower IL-10, Gata-3, IL-12 and IFNγ mRNA expression in $H_4R^{-/-}$ DCs as compared to WT controls. Among the genes tested, CFA treatment reduced the expression of IL-1ß, IL-4, IL-10, CCL19 and CCR7 significantly, regardless of the genotype of the cells. We also found genotype-dependent differences in response to CFA; CFA treatment reduced IL-6, Gata-3, and T-bet expression in WT animals, but it was ineffective in the H_4R deficient DCs. In KO mice IFNγ mRNA levels were elevated by *in vivo* CFA injection in contrast to WT animals where it remained unaltered (Figure 23. A-K).


relative TNF-alpha mRNA expression
0.06
0.05
0.04
0.03
0.02
0.01
0.00
PBS
CFA
A
relative IL-1 beta mRNA expression
1.2
1.0
0.8
0.6
0.4
0.2
0.0
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*
PBS
CFA
B
relative IL-6 mRNA expression
0.0035
0.0030
0.0025
0.0020
0.0015
0.0010
0.0005
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PBS
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D
relative IL-10 mRNA expression
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PBS
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E
relative Gata-3 mRNA expression
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0.016
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relative IL-12 mRNA expression
0.010
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relative IFN-gamma mRNA expression
0.0020
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0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
**
*
PBS
CFA
H
relative T-bet mRNA expression
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
*
PBS
CFA
I
relative CCR7 mRNA expression
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.00
***
***
PBS
CFA
J
relative CCL19 mRNA expression
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
***
**
PBS
CFA
K

Figure 23.

In vivo cytokine production of WT (■) and $H_4R^{-/-}$ (■) DCs

Cytokine responses of in vivo-activated WT (■) and $H_4R^{-/-}$ (■) DCs. WT and $H_4R^{-/-}$ mice were injected CFA containing stable emulsion (200 µl/mouse intra-peritoneally). After 7 days the TNF-α (A), IL-1ß (B), IL-6 (C), IL-4 (D), IL-10 (E), Gata-3 (F), IL-12 (G), IFNγ (H), T-bet (I), CCR7 (J), CCL19 (K) expressions of isolated splenic DCs were measured by real-time PCR Values are shown as means ± SEM of 6 independent experiments. Statistical significance was calculated using two-way ANOVA and Tukey as post hoc test. (: p<0.05, **: p<0.01, ***: p<0.001).*

Human studies

1. Histamine modulates monocyte-derived DC differentiation

Differentiation to IDCs is a complex, highly regulated process to which several mediators can contribute. In our human studies first we tested how histamine, potentially acting through different HRs can modulate the differentiation of the previously identified $CD1a^-$ and $CD1a^+$ DC subsets. Monocyte-derived DCs were generated by IL-4 and GM-CSF cytokine combination in the presence or absence of histamine applied in the course of cell differentiation on day 0, 2 and 5. Flow cytometric measurements revealed that histamine significantly reduced the proportion of $CD1a^+$ DCs (Figure 24.A) as compared to cells generated in the absence of histamine. To identify the HR involved in this effect, specific pharmacological blocking agents (all used at 10 µM concentration) were used during the differentiation process. We found that the presence of the H_2R antagonist famotidine could prevent the reducing effect of histamine on $CD1a^+$ cell differentiation (Figure 24.A, B). The H_1R antagonist pyrilamine further reduced the ratio of $CD1a^+$ cells, whereas both H_4R antagonists had no or only marginal effects (Figure 24.A). The inhibitory effect of histamine on $CD1a^+$ cell differentiation was confirmed at the protein level by flow cytometric analysis showing the cell surface expression of H_2R on $CD1a^-$ and $CD1a^+$ DC subsets and also the efficient blocking of this effect by famotidine, but not by the other inhibitors (Figure 24.B).

These results show that histamine exerts a subset-specific effect on the differentiation of the DC subsets differing in their CD1a expression. As famotidine was the only inhibitor that could restore $CD1a^+$ DC generation, whereas the H_1R antagonist pyrilamine and the H_4R blockers JNJ7777120 and JNJ10191584 had no such effect, these results suggest that H_2R is preferentially responsible for this histamine-mediated effect.

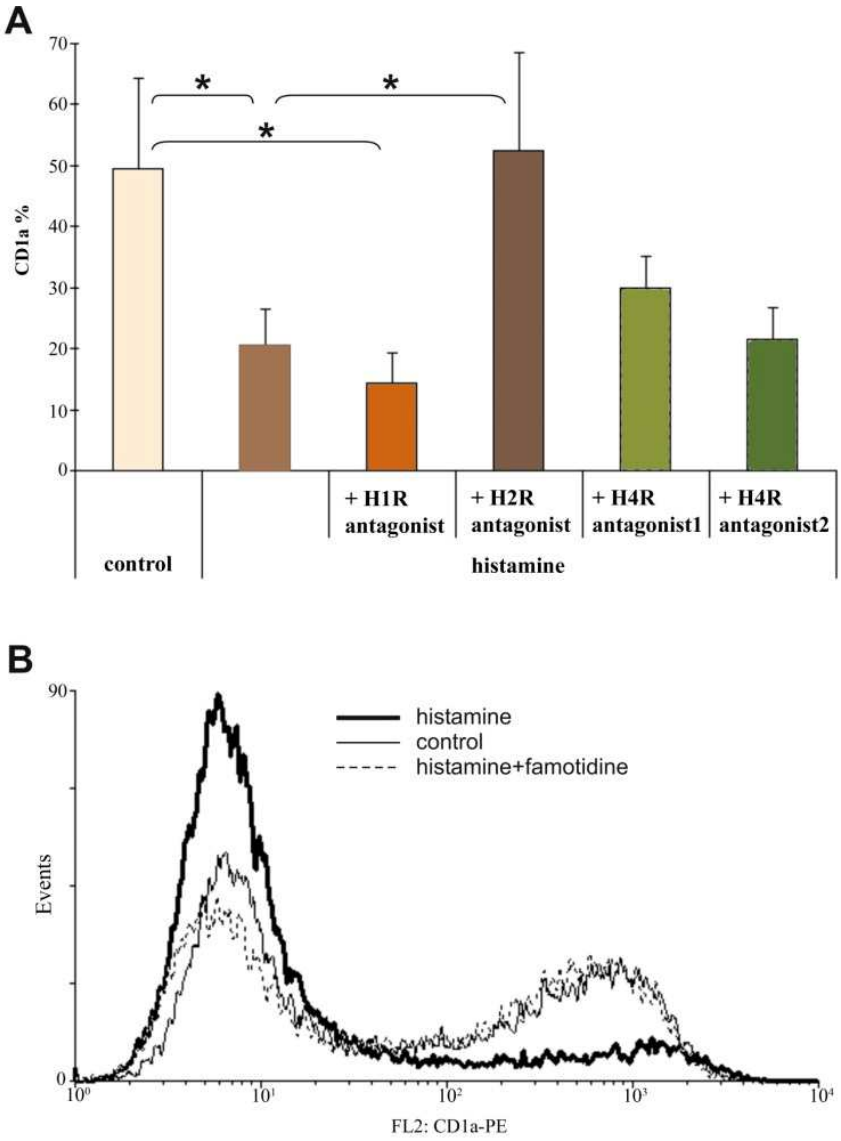


Figure 24.

The effect of histamine and HR antagonists on the development of $CD1a^-$ and $CD1a^+$ monocyte-derived DC subsets

The percentage of $CD1a^+$ cells was detected by flow cytometry in in vitro generated resting monocyte-derived DCs, in DCs differentiated in the presence of histamine and in DCs differentiated in the presence of histamine in combination with the H_1R antagonist pyrilamine, the H_2R antagonist famotidine, or the H_4R antagonists JNJ7777120 and JNJ10191584 (all in 10 μM concentration) (A). Mean ± SD of 3 independent experiments are presented. The histograms show the distribution of $CD1a^-$ and $CD1a^+$ cells generated in the presence or absence of histamine or in the presence of histamine and famotidine (B).

2. Expression of HRs in $CD1a^-$ and $CD1a^+$ DC subsets

The expression of HRs by human DCs has previously been demonstrated [56] [57] [58] [59] [60] [61] however, their expression in the phenotypically and functionally distinct

CD1a$^-$ and CD1a$^+$ DC subsets has not been investigated so far. To assess the DC subset-specific expression of HRs in *in vitro* differentiated LPS+IFNγ-activated monocyte-derived DCs obtained from 9 healthy individuals with an average of 46±11 % CD1a$^+$ ratios, the cells were sorted to CD1a$^-$ and CD1a$^+$ subpopulations by flow cytometry and H_1R, H_2R and H_4R expression was compared by QRT-PCR. H_3R expression could not be detected, H_1R expression was significantly higher in CD1a$^-$ DCs, but H_4R was higher in CD1a$^+$ cells as compared to their CD1a$^-$ counterpart, whereas H_2R was expressed by both subsets at high levels (Figure 25.A). This finding was also supported by the detection of H_2R by flow cytometric analysis on the surface of both CD1a$^-$ and CD1a$^+$ cells at comparable levels (Figure 25.B).

These results revealed the subset-specific expression of H_1R and H_4R and identified H_2R as the dominant HR in human monocyte-derived DCs irrespective of their subset.

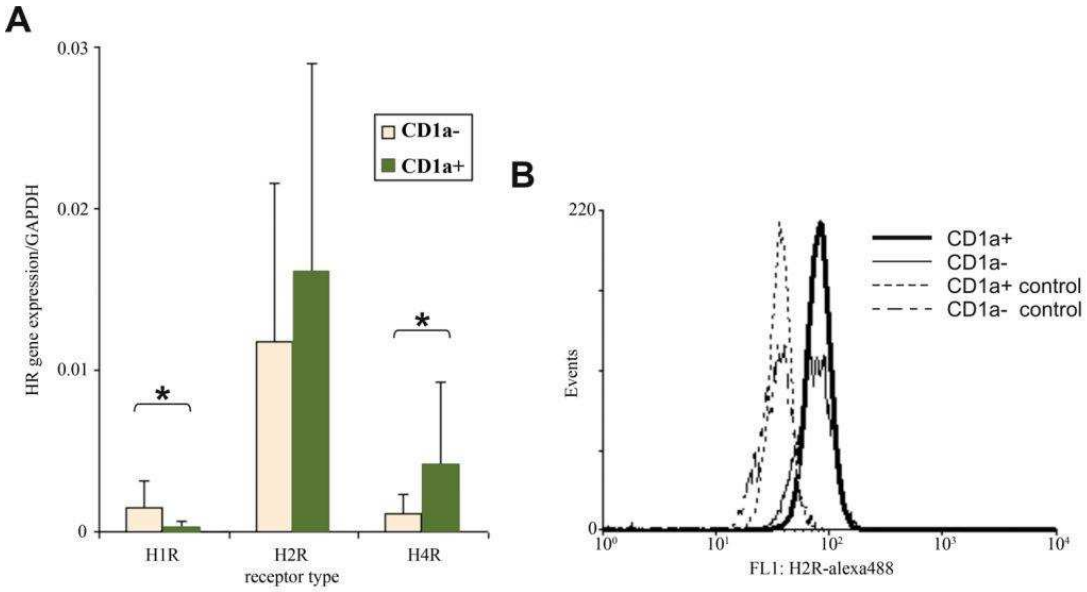


Figure 25.

Gene expression profiling of HRs in monocyte-derived DC subsets

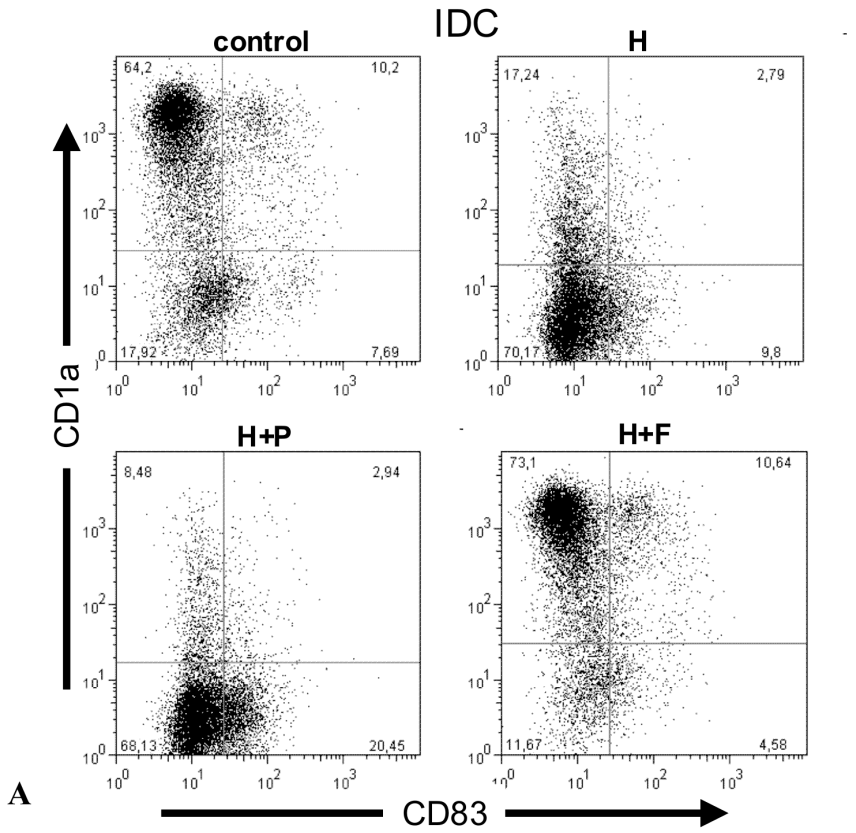
DCs were generated with the differentiating cytokines IL-4 and GM-CSF on day 0 and 2 of in vitro cultures. On day 5 the cells were stimulated by LPS+IFNγ for 24 hours. A fraction of cells was left untreated for using it as a non-activated DC control. Resting and activated DCs were harvested on day 5 and 6, respectively and subjected to RNA isolation and reverse transcription to prepare cDNA. Gene expression of HRs was measured in DCs isolated from 9 different donors all sorted to CD1a$^-$ and CD1a$^+$ DC fractions (A). QRT-PCR was performed in triplicates and the comparative C_t method was used to quantify transcripts relative to the endogenous house keeping gene GAPDH. Expression of H_2R was detected by flow cytometry using indirect staining (B).

3. Effect of histamine on the activation of CD1a$^-$ and CD1a$^+$ DC subsets

Previously we described that inflammatory cytokines can block the transition of CD1a$^-$ cells to CD1a$^+$ DCs and thus stabilize the CD1a$^-$ phenotype for further activation [74]. To monitor the effect of histamine on the activation of CD1a$^-$ and CD1a$^+$ subsets, DCs were generated in the presence of histamine and activated by the combination of LPS and IFNγ. Cell surface expression of CD1a and the activation marker CD83 was monitored in the CD1a$^-$ and CD1a$^+$ DC subsets by flow cytometry. The results summarized in Figure 26.A-C show that histamine did not affect significantly the expression of CD83, and both the CD1a$^-$ and CD1a$^+$ subsets could be activated by LPS+IFNγ. Histamine, present in the course of *in vitro* monocyte-derived DC differentiation, was able to reduce the proportion of CD1a$^+$ cells (Figure 24.) and consequently decreased the ratio of both resting and activated CD1a$^+$CD83$^+$ cells, while the CD1a$^-$CD83$^+$ fraction became larger only in mature DCs (Figure 26.A-C). In line with our results obtained with resting DCs the H_2R antagonist famotidine suspended this histamine-mediated effect (Figure 26.A-C), while the H_1R antagonist pyrilamine further reduced the ratio of CD1a$^+$CD83$^+$ cells. These results suggest that histamine modulates DC activation through its inhibitory effect on CD1a$^+$ DC differentiation.

DCs are sensitive to changes in their environment and thus can be activated by various stimuli, which result in the secretion of cytokines and chemokines [81]. Our previous results demonstrated that upon activation by CD40L or TLR ligands, the CD1a$^-$ and CD1a$^+$ DCs secrete different sets of cytokines [74]. To test whether histamine or HR-specific antagonists can modify the cytokine production of DC subsets we measured the concentrations of cytokines in the culture supernatant of activated CD1a$^-$ and CD1a$^+$ DC cultures by ELISA. Statistically significant increase of the secreted pro-inflammatory cytokine IL-6 (Figure 26.D) and the regulatory cytokine IL-10 (Figure 26.E) could be shown when DCs were generated in the presence of histamine. This effect also could be inhibited by famotidine indicating again the involvement of H_2R in this process. The secretion of IL-12p70 was detected preferentially in DCs with high, >60% of CD1a$^+$ cell content and its production could not be consistently inhibited by famotidine.

These results demonstrate that the presence of histamine results in a decreased proportion of the CD1a$^+$ DC subset and this has a significant impact on the production of IL-6 and IL-10 cytokines. As CD1a$^+$ DCs have previously been identified as potent producers of IL-12p70, we propose that the increase of IL-10 secretion can at least partially be the consequence of altered cytokine balance in the DC microenvironment.


IDC
control
H
H+P
H+F
64,2
10,2
17,92
7,69
17,24
2,79
70,17
9,8
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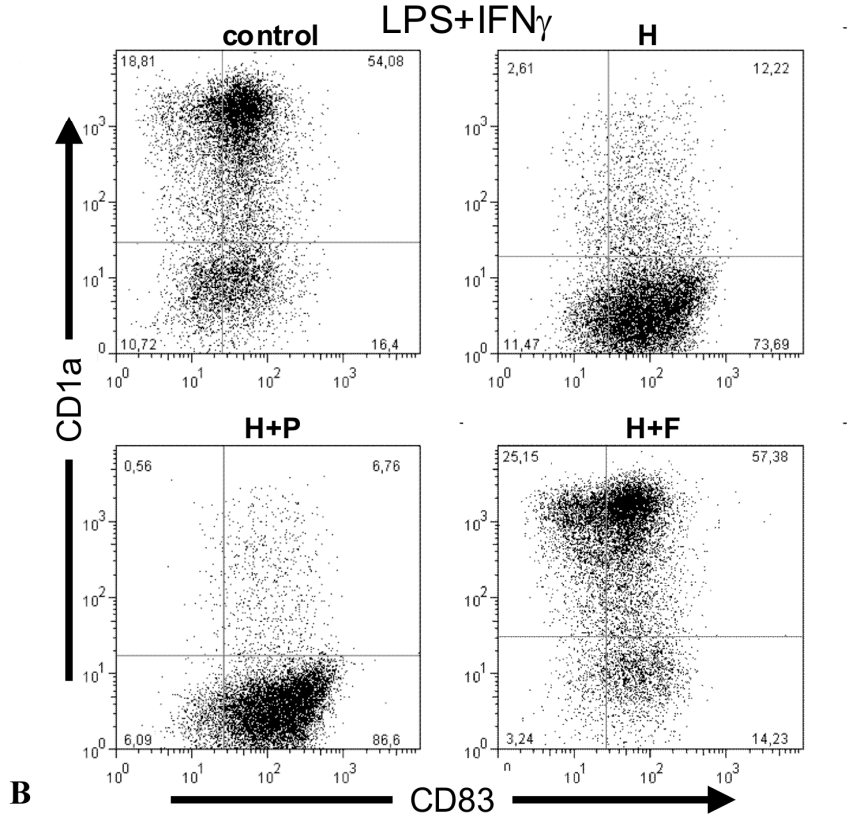
2,94
68,13
20,45
73,1
10,64
11,67
4,58
CD1a
CD83

A


LPS+IFNγ
control
H
H+P
H+F
18,81
54,08
10,72
16,4
2,61
12,22
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25,15
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14,23
CD1a
CD83

B

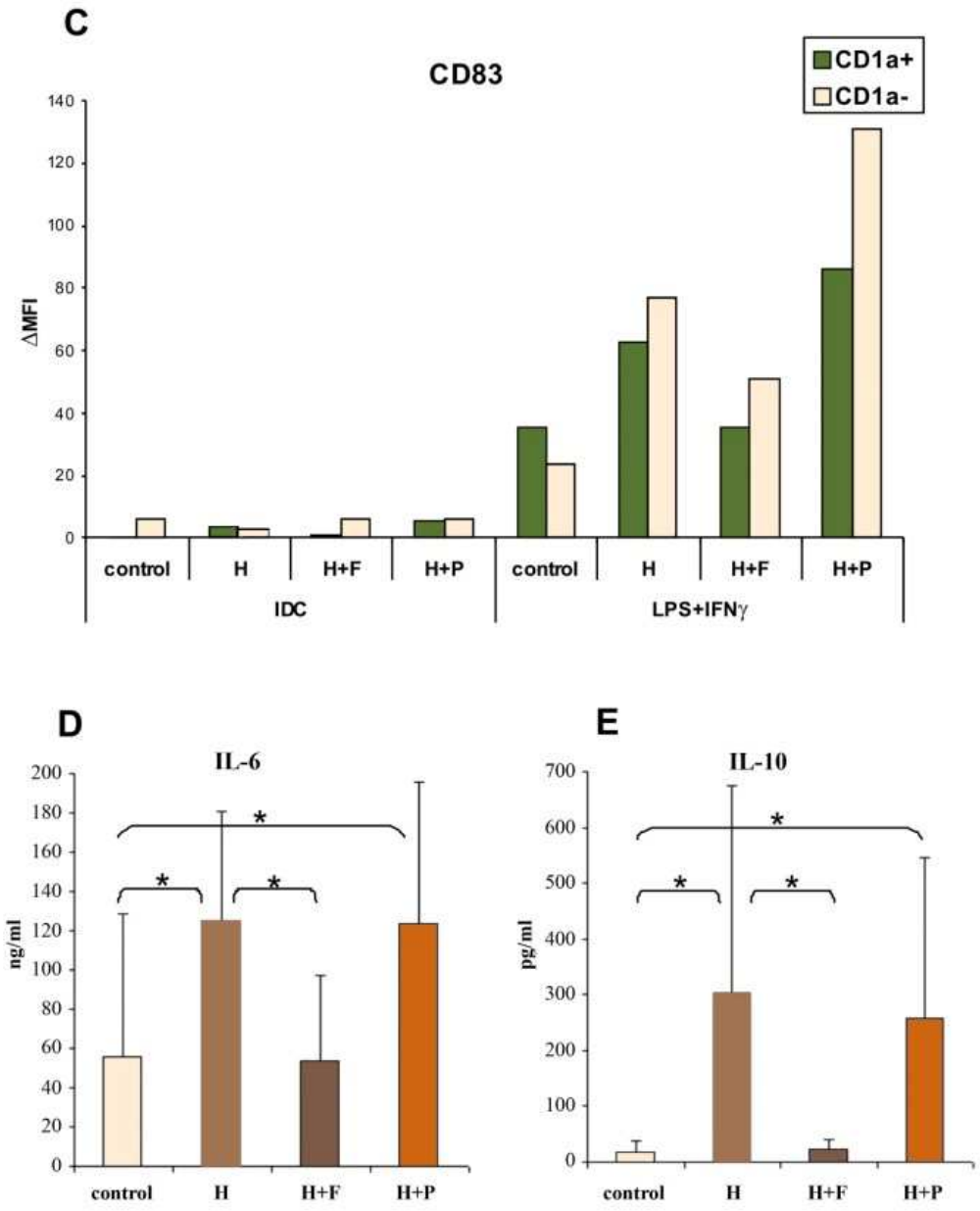

Figure 26.

Effect of histamine on the induction of monocyte-derived DC activation

DCs were differentiated in the presence of histamine as described in Figure 23. then the differentiated cells were activated by LPS+IFNγ on day 5 of culture. Cells were harvested on day 5 and 6 and the expression of CD83 was measured on the surface of immature (IDC) and LPS+IFNγ-activated CD1a$^-$ and CD1a$^+$ cells (A – C). F and P refer to famotidine and pyrilamine, respectively. The histamine-modulated cytokine secretion by activated DCs was monitored by measuring the concentrations of IL-6 (D) and IL-10 (E) cytokines in the culture supernatants of resting and activated DCs differentiated in the absence or presence of histamine or histamine together with the H_2R antagonist famotidine. Mean ± SD of triplicate measurements performed with DCs of 6 independent experiments are shown.

4. Effect of histamine on DC migration

Migration is an important and indispensable feature of DCs. IDCs migrate to both body surfaces and interstitial spaces where they easily make contact with self- or foreign antigens. Histamine not only induces cell migration mediated by different HRs [59] [60] [61], but at the same time it is also able to stimulate matrix degrading enzyme production [82]. Migration of DCs in blood and lymph, and from peripheral tissues to secondary lymphoid organs is regulated at multiple levels [16] [83]. Our *in vitro* migration experiments revealed that the presence of histamine during monocyte-derived DC differentiation significantly enhanced the migratory potential of DCs that could be shown at the level of both spontaneous and MIP-3β-induced migration (Figure 27.A). Famotidine, the specific H_2R antagonist could reverse this effect (Figure 27.B) pointing to the role of H_2R in the modulation of cell mobility. Matrix degrading enzymes are also essential components of cell migration. Monitoring mRNA expression of MMP-9 and MMP-12 by QRT-PCR showed that the presence of histamine was able to up regulate the mRNA expression of these matrix degrading enzymes in both resting (IDC) and activated (MDC) DCs. This effect could also be reversed by famotidine further supporting the role of H_2R in regulating DC migration (Figure 27.C, D).

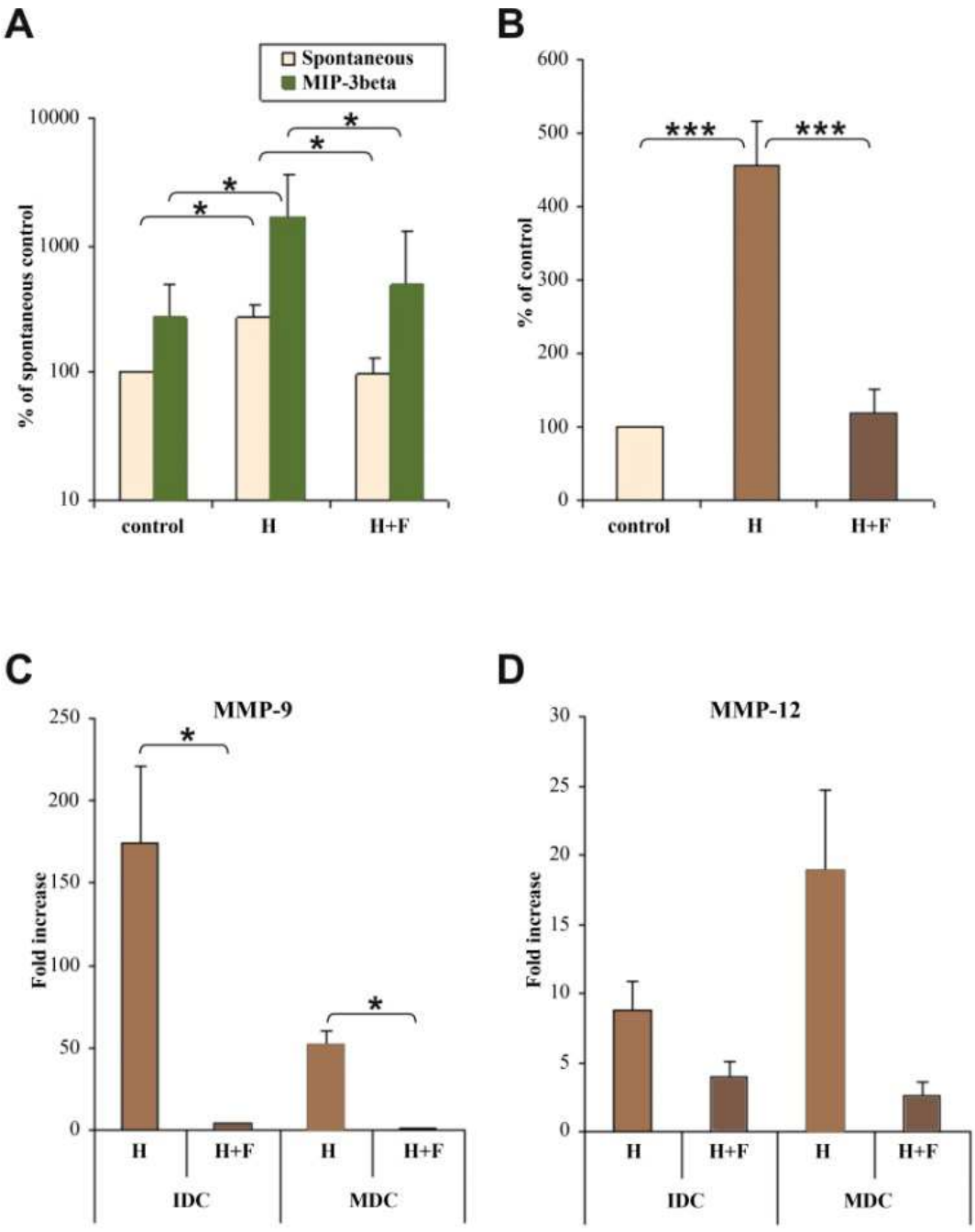

Figure 27.

The effect of histamine on monocyte-derived DC migration

Migration of activated DCs generated with or without histamine was measured in the presence or absence of MIP-3β chemokine by Transwell system (A, B). The effects of histamine on the expression of matrix metalloproteinases MMP-9 and MMP-12 was determined by measuring relative mRNA expression levels in IDCs and MDCs by QRT-PCR (C, D). Mean ± SD of five independent experiments is shown.

5. Histamine-induced expression of C5a receptor is restricted to the CD1a$^-$ DC subset

In the next set of experiments we performed a global screening using a Q-PCR-based array to identify chemokines and chemokine receptors involved in the histamine-induced H_2R-mediated enhancement of DC migration. Following LPS+IFNγ activation, the monocyte-derived DCs generated in the presence of histamine, famotidine, or histamine together with famotidine were separated to CD1a$^-$ and CD1a$^+$ subsets and their chemokine and chemokine receptor expression profiles were determined. When DCs were generated in the presence of histamine, increased mRNA expression of C5aR1 was detected in the CD1a$^+$ subset and famotidine abolished this effect (Figure 28.A). However, C5aR1 protein expression clearly showed that its expression was dramatically upregulated by histamine preferentially in the CD1a$^-$ subset at both their resting (Figure 28.B) and activated (Figure 28.C) differentiation states. As famotidine could completely abolish the effect of histamine, these results also revealed the H_2R dependency of this histamine-induced effect.

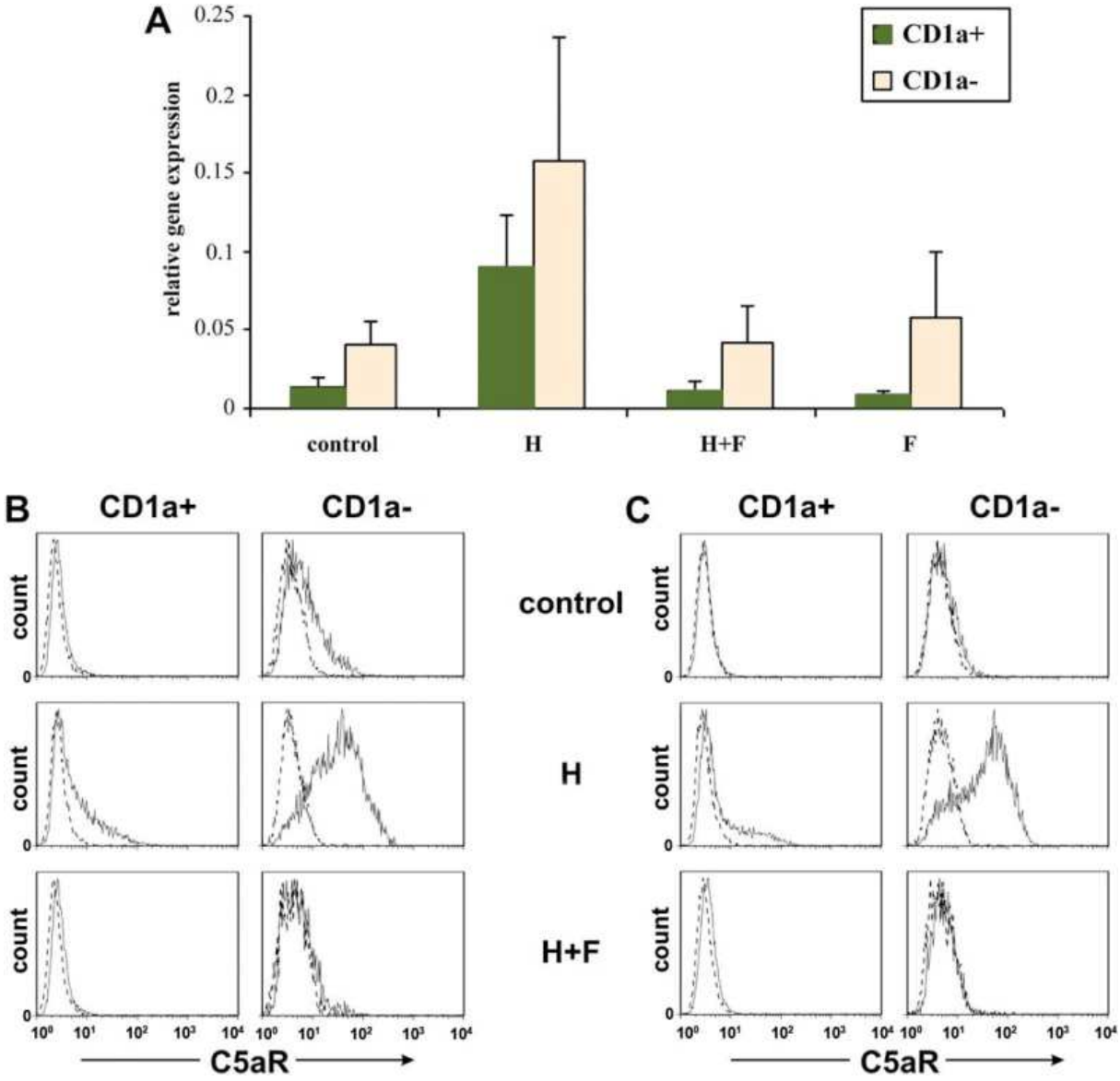

Figure 28.

The effect of histamine on the expression of C5aR1 gene and protein

Resting and activated monocyte-derived DCs, differentiated in the presence or absence of histamine or in the presence of histamine in combination with famotidine, were sorted to CD1a$^-$ and CD1a$^+$ DC subsets. The subset-specific expression of C5aR1 in the CD1a$^-$ and CD1a$^+$ DCs was determined at both mRNA (A) and protein (B, C) levels measured by a Q-PCR array and by flow cytometry, respectively. Mean ± SD of 3 independent experiments is shown.

In addition to identifying C5aR1 as a histamine-induced chemotactic receptor of DCs preferentially expressed by the CD1a$^-$ subset, we also identified further migration-related genes as summarized in Table 6. The expression of CXCR4 and CX3CR1 also increased significantly in DCs generated in the presence of histamine and this effect on CX3CR1 could

be inhibited by famotidine in the CD1a$^+$ DC subset, while in CD1a$^-$ cells histamine did not exert this modulatory effect.

CD1a$^+$ DC

Gene	*Compared groups*	*Fold change*	*p value*
C5aR1	activated CD1a$^-$/CD1a$^+$ DC	2.92	0.177
CXCR4		0.87	0.573
CX3CR1		1.55	0.344
C5aR1	differentiated in the presence/absence of histamine	6.54	0.088
CXCR4		2.89	**0.016**
CX3CR1		4.04	**0.015**
C5aR1	differentiated in the presence of histamine/histamine+famotidine	8.00	0.08
CXCR4		1.47	0.310
CX3CR1		3.95	**0.024**
C5aR1	differentiated in the presence of histamine+famotidine/famotidine	1.23	0.726
CXCR4		1.00	0.992
CX3CR1		0.90	0.878

Table 6.

Relative expression of chemokine receptor genes in monocyte-derived DC subsets modulated by histamine through H_2R

CD1a$^-$ and CD1a$^+$ monocyte-derived DCs were differentiated and treated as described in Figure 27. Comparison of the differentially treated DC samples was expressed as fold increase of mRNA levels in CD1a$^+$ versus CD1a$^-$ cells.

7. DISCUSSION

DCs represent a minor population of leukocytes but act as important sentinels and are characterized by diverse biological functions. Beside capturing, processing and presenting antigens, they are capable of regulating the direction, quantity and quality of effector mechanisms that serve for the elimination of invading organisms and dangerous self structures [14] [23]. Through the activation of antigen-specific T lymphocytes and the production of cytokines and chemokines DCs act as a functional bridge between innate and acquired immunity.

In this PhD work we investigated the functional cross-talk of histamine with DCs in two experimental systems. The murine system offered means to compare the effects of histamine on DCs in genetically modified strains *in vivo* and by using tissue-derived DCs *in vitro*. The human system offered us to analyse the effects of histamine on DC subtype differentiation and functions.

The size of the HRs has been shown to vary in a wide range depending on the tissue, cell type, post-translational modification and detection method [79] [80] [84]. In our hands immunoblot (Figure 8.) and flow cytometric analysis (Figure 9.) certified the expression of H_1R, H_2R and H_4R in mouse splenic DCs. Beside the H_4R protein band detected by Western blot the other higher molecular mass band could correspond to the glycosylated form of the receptor.

DC differentiation is a complex process regulated at multiple levels. We observed no change in the expression of H_1R, whereas that of the H_2R and H_4R decreased significantly in the course of *in vitro* differentiation of bone marrow-derived DCs (Figure 10.A, B and C). LPS did not alter H_1R or H_2R protein expression, however H_4R expression was elevated significantly by LPS stimulation (Figure 10.D, E and F). It was demonstrated that during *in vitro* differentiation of DCs from human monocytes the expression of H_1R and H_3R reduced, while that of the H_4R increased [58] [61]. Others showed higher expression of H_4R in resting than in activated $CD4^+$ and $CD8^+$ T lymphocytes [47]. Based on these data we suggest, that since different HRs activate distinct signaling cascades, the observed alterations in HR expression may modify the strength and the direction of histamine-mediated effects depending on the life cycle or activation state of the cells.

Antigen presentation is of crucial importance in the activities of DCs, still a few data are available on the effects of histamine on basic DC functions. We found significantly

decreased antigen presentation of murine splenic DCs in the presence of histamine (Figure 11.A). We identified H_4R as the regulator of this process, as its agonist (4-MH) similarly to histamine declined the peptide presenting function of DCs (Figure 11.B), while the H_4R antagonist JNJ10191584 almost completely reversed this effect (Figure 12.A). The observation that significantly higher amounts of IL-2 were produced when the 5/4E8 T cell hybridoma cells were stimulated by peptide-loaded DCs of H_4R-deficient mice than DCs from WT controls however, further indicated the role of H_4R in this histamine-mediated effect (Figure 12.B). We also found that long-term H_4R deficiency had a more pronounced impact on antigen presentation than short-term antagonist treatment. These data suggest that histamine exerts this effect through H_4R and it can evolve both acutely during antigen presentation and also in the course of DC development.

The efficacy of antigen presentation can be influenced by several factors, such as the subtype of DCs, the mechanism of antigen uptake and processing and the expression of cell surface costimulatory molecules. The molecular mechanism, by which histamine acts on antigen presentation via H_4R remains unknown, since we could not detect any differences in either MHC class II or costimulatory molecule expression of histamine-treated $H_4R^{-/-}$ and WT splenic or differentiated DCs (Figure 13. and 14.). IFNγ has been shown to affect certain components of the antigen presentation machinery [85] [86]. Since we found that the induction of IFNγ production was more pronounced in H_4R-KO DCs as compared to the WT ones (Figure 23.H), enhanced IFNγ levels may account for the elevated T cell-stimulatory capacity of H_4R-deficient DCs.

Several factors including chemokines, cytokines and small bioactive molecules like histamine may influence the migratory and chemotactic properties of DCs and histamine has long been known to influence the migration of DCs [47]. Before the discovery of H_4R chemotactic effects provoked by histamine were attributed primarily to H_1R [87] and H_2R [67] [88]. Later, the migration regulatory effect of histamine through H_4R of different immune cell types such as eosinophils [89], mast cells [90], γδ T cells [91] and natural killer cells was demonstrated [59]. The involvement of H_2R and H_4R was shown in human DCs [61], while in murine differentiated DCs the role of H_1R and H_4R was shown [70].

In our migration studies we found that neither different concentrations of histamine nor 4-MH could induce a significant alteration in splenic DC migration *in vitro* (Figure 15.A and B). Supposing that spleen-derived DCs are less susceptible to acute histamine stimulus than freshly differentiated cells, we monitored the effect of a long-term histamine treatment on DC migration. We found that the length of histamine treatment during *in vitro* DC

differentiation correlated to migrating cell numbers (Figure 15.C). Comparing the migratory capacity of WT and $H_4R^{-/-}$ DCs we concluded that DCs generated in the absence of H_4R possessed a significantly reduced migration capacity then WT cells, suggesting the role of H_4R in this process (Figure 16.). Up to now only one paper has been published in which the effect of histamine on mouse DC migration via H_1R and H_4R was demonstrated [70]. However, we have to notice that in most cases histamine and HR ligands were applied in much lower concentration than the above (1mmol/l), physiologically not available concentration.

Adhesion is an indispensable step of cell migration. We were the first to use an impedance-based xCELLigence RTCA SP system to study the adhesive capacity of murine differentiated DCs. Histamine significantly increased DC adhesion when presented transiently or for a long period of time (Figure 17. and 18.). We identified H_4R as the receptor responsible for this histamine-mediated effect, as comparison of the adhesive capacity of WT and $H_4R^{-/-}$ DCs revealed that both parameters characterizing the adhesion of the cells, i.e. slope and ΔCI, were significantly higher in H_4R-KO than in WT cells (Figure 20.). These results were surprising since previously we detected impaired migration of H_4R-deficient DCs. As adhesion is a prerequisite of cell migration, the above processes were expected to show direct correlation. It has recently been published that murine DC migration could be negatively regulated by adhesion [92]. The involvement of H_4R was further confirmed by *in vitro* H_4R antagonist treatment showing that JNJ10191584 could effectively reduce the increasing effect of histamine on DC adhesion (Figure 21.). Further experiments are required to obtain more molecular data on the action of histamine via H_4R governing adhesion and migration however, these results revealed the H_4R dependency of this histamine-induced effect.

Cytokines and chemokines secreted by activated DCs are crucial components of the modulation of immune processes. The repertoire of soluble cytokines produced by DCs is associated with the developmental stage or maturation status of the cells and are important in inducing an effective primary immune response [93] and several lines of evidence support that histamine influences cytokine production of DCs [37] [66]. Before the discovery of H_4R, mainly H_2R was found to be involved in histamine-induced Th2 polarization of DCs [37] [60] [66].

Increasing body of evidence support that Toll-like receptor signals in DCs not only enhance the endocytosis of antigens but also influence the immunological outcome of their interactions with T cells. The pro-inflammatory cytokine IL-1ß is a crucial mediator of the

innate immune system and beside activated mononuclear phagocytes, DCs are also important sources of this cytokine. Mazzoni *et al*. have shown that histamine blocked LPS-induced IL-1ß expression in immature human DCs [66]. In this experimental system the general receptor activator histamine was used, thus the results gained are possibly reflect the accumulated effect on different HRs.

In our work IL-1ß mRNA expression was tested using either H_4R agonist or antagonist treatment followed by LPS stimulation. Our results revealed that the LPS-induced expression of IL-1ß was further induced by the H_4R agonist 4-MH, while the H_4R antagonist JNJ7777120 effectively reversed this effect demonstrating the participation of histamine via H_4R in governing IL-1ß expression (Figure 22.A).

Most data on DC cytokine production is based on the results of *in vitro* studies. In order to get more insight into its importance, we applied an *in vivo* assay in which WT and $H_4R^{-/-}$ animals were stimulated by CFA and then the cytokine profile of DCs was compared. $H_4R^{-/-}$ DCs showed significantly altered mRNA expression of some Th1 and Th2 cytokines. Most papers based on *in vitro* data report the elevation of Th2 and reduction of Th1 cytokines, which mediated principally through H_2R [60] [94]. Gutzmer and co-workers detected reduced IL-12p70 production in human monocyte-derived DCs, while IL-10 expression was not altered after histamine treatment. This effect was mediated by H_2R and H_4R [61]. In line with previous data we could not detect any changes in TNF-α expression (neither after treatment nor in the genetically different mice) (Figure 23.A) [55]. CFA treatment showed genotype-dependent effects in case of some molecules; it decreased IL-6, Gata-3 and T-bet mRNA expression in WT DCs, while it proved to be inefficient in $H_4R^{-/-}$ DCs (Figure 23.C, F and I). Our results on IL-6 are contradictory to the data published by Dunford *et al.* [55]. Applying different activation stimuli *in vitro*, they measured decreased IL-6 protein expression after H_4R blockade. $H_4R^{-/-}$ DCs produced more IFNγ than WT ones following CFA treatment (Figure 23.H). We detected similar difference in IFNγ expression when comparing WT and $HDC^{-/-}$ DCs [71].

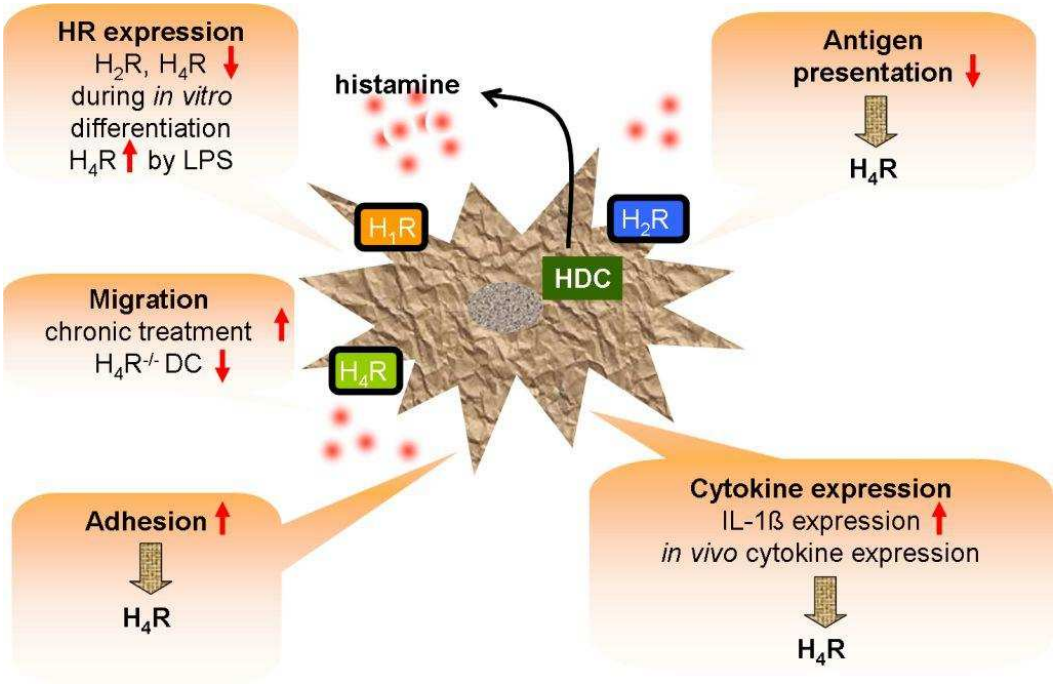

Figure 29.

Summary of the results observed in the murine system

DCs expressed H_1R, H_2R and H_4R. The expression level of H_2R and H_4R reduced in the course of in vitro differentiation and LPS could significantly enhance H_4R expression. Histamine affected DC activities, namely the antigen presentation, migration, adhesion and cytokine expression were influenced by histamine via H_4R.

In the second part of the studies we investigated the effects of histamine on the differentiation and activities of two, developmentally related and *in vivo* relevant human monocyte-derived DC subsets generated from $CD14^{high}$ monocytes [74]. We found that i) histamine modulated DC differentiation by supporting the development of $CD1a^-$ DCs; ii) this effect was attributed to H_2R as only its specific synthetic inhibitor could restore the generation of $CD1a^+$ cells; iii) H_2R was highly expressed by both DC subsets, whereas H_1R and H_4R were expressed in a subset-specific manner; iv) histamine modulated DC activation through its inhibitory effect on $CD1a^+$ DC differentiation; v) histamine-induced reduction of $CD1a^+$ DCs resulted in increased secretion of IL-6 and IL-10; vi) histamine modulated the expression of C5aR1 in a H_2R and subset specific manner; vii) histamine induced spontaneous and chemokine-mediated DC migration of both subsets and modulated the production of the MMP-9 and MMP-12 enzymes also involved in the regulation of DC migration.

DC differentiation is a complex and highly regulated process driven by the actual tissue environment of the cell. Lipids and lipoproteins have previously been identified as modulators of the cell surface expression of type I and type II CD1 proteins, which act as both presenting molecules of microbial glyco- and phopholipids and also as phenotypic markers of human DC subsets [95]. The modulatory effect of histamine on the expression of CD1a and the *in vitro* differentiation of DCs has previously been reported [65]. Here we not only confirmed these findings, but extended our studies to demonstrate that histamine has an impact on other DC functions which may be regulated in a subset-specific manner.

DC functions may be associated with more than one HR, thus we sought to generate monocyte-derived DCs *in vitro* in the presence of histamine or histamine in combination with pharmacological antagonists of H_1R, H_2R and H_4R. As famotidine, a potent antagonist of H_2R was the only compound that could prevent the histamine-mediated inhibition of $CD1a^+$ DC differentiation we identified H_2R as the receptor involved in this regulation (Figure 24.).

Several research groups demonstrated the expression HRs in human DCs [56] [57] [58] [59] [60] [61] however, their expression in $CD1a^-$ and $CD1a^+$ cells has not been analyzed so far. Similar to previous results [96] we could not detect H_3R expression in DCs but found high expression of H_2R in both $CD1a^-$ and $CD1a^+$ DCs. Interestingly, H_1R and H_4R mRNA expression showed a subset-dependent pattern: $CD1a^-$ DCs expressed significantly higher levels of H_1R than $CD1a^+$ cells, whereas H_4R expression was significantly higher in the $CD1a^+$ DC subset (Figure 25.A). This finding raises interesting questions concerning the role of H_4R in a situation where due to the presence of histamine the generation of the cell type i.e. $CD1a^+$ DCs carrying the receptor at high levels is inhibited. Based on this scenario and taken the previously described inflammatory nature of $CD1a^+$ DCs in individuals with high $CD1a^+$ DC ratios may develop more severe inflammation in the presence of histamine than those with low $CD1a^+$ numbers. Alternatively, this expression pattern may have a regulatory function to keep histamine-mediated regulation under check.

DC maturation is initiated by the engagement of different surface receptors and results in phenotypic and functional changes. Histamine was shown to induce transient up-regulation of MHC class II proteins and the costimulatory molecules CD40, CD80 and CD86 in human monocyte-derived DCs and antagonists of both H_1R and H_2R prevented histamine-induced CD86 expression [57] but did not modify CD40 expression [66]. When DCs were activated with LPS+IFNγ we found that due to the histamine-induced reduction of $CD1a^+$ DC numbers the ratio of $CD1a^+CD83^+$ cells decreased, while the $CD1a^-CD83^+$ cell fraction became more prominent (Figure 26.A and B). This observation demonstrates that histamine, by

counteracting $CD1a^+$ DC differentiation has an impact on the DC subset distribution of mature DCs in a H_2R-dependent manner.

The hallmark of activated DCs is the production of cytokines and chemokines, which may act in an autocrine or paracrine manner. Previous results demonstrated that histamine induced altered cytokine production in DCs, supported polarization of T lymphocytes to Th2 direction [57], and the cytokine profile of resting $CD1a^-$ and $CD1a^+$ DCs was different [97]. In line with these findings we also observed that upon activation by CD40L or TLR ligands both $CD1a^-$ and $CD1a^+$ DCs secreted pro-inflammatory cytokines but IL-12p70 and CCL1 production was mainly attributed to the $CD1a^+$, whereas IL-10 secretion to the $CD1a^-$ subset [74]. DCs generated in the presence of histamine and activated by LPS+IFNγ produced significantly higher levels of the pro-inflammatory cytokine IL-6 and the regulatory cytokine IL-10 as compared to cells generated in the absence of histamine (Figure 26.D and E). Again, famotidine was the only inhibitor that could interfere with this effect. In our studies IL-12p70 secretion was detected in DCs with high (>60%) $CD1a^+$ cell content and famotidine had no significant effect on cytokine secretion indicating that IL-12p70 secretion is not dependent on H_2R. In a previous study IFNγ was shown to up-regulate H_4R and its stimulation resulted in the down-regulation of IL-12p70 and CCL2 production in human monocyte-derived DCs [63].

Resting DCs migrate to both body surfaces and interstitial spaces where they encounter with self- or foreign antigens. After antigen challenge, migration of activated DCs through lymphatics ensures DC-T cell contact in lymphoid organs. Several independent research groups found that histamine [59] [61] or histamine agonists [60] [61] exerted chemotactic effects for resting, but not for activated DCs [60]. By using specific receptor antagonists the involvement of both H_3R [59] [60] and H_1R [60] has been demonstrated as modulators of cell migration. In a skin DC migration assay both histamine and a H_4R agonist induced enhanced chemotaxis, which could be blocked by both H_1R and H_4R antagonists. Similar results were obtained *in vitro* by using bone marrow-derived mouse DCs [70]. Our previous results also verified the role of H_4R in murine DC migration [98].

In our present migration experiments histamine increased both spontaneous and MIP-3β-induced migration of DCs. Famotidine was the only HR antagonist that could reverse this effect (Figure 27.A and B). Histamine was also shown to stimulate the production of the matrix degrading enzymes and the histamine-induced increase of MMP-13 and MMP-3 production was shown *in vitro* (Figure 27.C and D) [82]. The essential role of MMP-9 in DC migration was measured in reconstituted basement membrane (Matrigel) and *in vivo* by

migration to the draining lymph node [99] and the expression of MMP-12 in DCs was also shown [100]. Histamine induced up-regulation of MMP-9 and MMP-12 and their inhibition by the H_2R blocker famotidine confirmed the dominant role of H_2R in this DC function as well.

Migration of DCs in blood and lymph, and from peripheral tissues to secondary lymphoid organs is regulated at multiple levels [16] [83] and a number of cytokines, chemokines and their receptors are implicated in the regulation of this complex process. The combination of chemokine receptors, induced in activated DCs when developed in the presence of histamine and stimulated with LPS+IFNγ involved C5aR1, CXCR4 and CX3CR1, previously identified in the sulpho-LacNAc expressing DCs (slanDCs/MDC-8), which represent the largest population of blood DCs (Table 6.) [101]. These circulating DCs are highly pro-inflammatory due to their production of TNF-α and IL-12p70, but in contrast to $CD1c^+$ conventional blood DCs they are negative for the CD11c, CD14 and CD1 markers. C5a is a pro-inflammatory mediator that has recently been detected in monocyte-derived DCs [102] and signaling through C5aR1 has been found to regulate the development of Treg and Th17 cells [103]. Here, we show for the first time that monocyte-derived DCs, differentiated in the presence of histamine and activated by LPS+IFNγ, preferentially give rise to $CD1a^-$ $C5aR1^+$ cells (Figure 28.).

CXCR4 endowed with potent chemotactic activity is up-regulated upon DC activation induced by LPS, TNF-α or CD40L [18] and engagement by its specific ligand CXCL12/stromal-cell derived factor-1 (SDF-1a) promotes DC activation, survival and chemotaxis both *in vitro* and *in vivo* [104]. The CX3CR1 transmembrane protein has been suggested to be enhanced by IFNγ in TNF/iNOS-producing DCs differentiated from classical monocytes [105] and it is involved in the recruitment of DCs by its specific ligand CX3CL1/fractalkine [106]. Based on these results we propose that histamine, in combination with inflammatory signals is able to induce the expression of a typical combination of chemokine receptors that also modulate monocyte-derived DC functions. However, this effect can be further up-regulated in the presence of the CCL19/MIP-3β chemokine, which specifically attracts CCR7 expressing activated DCs.

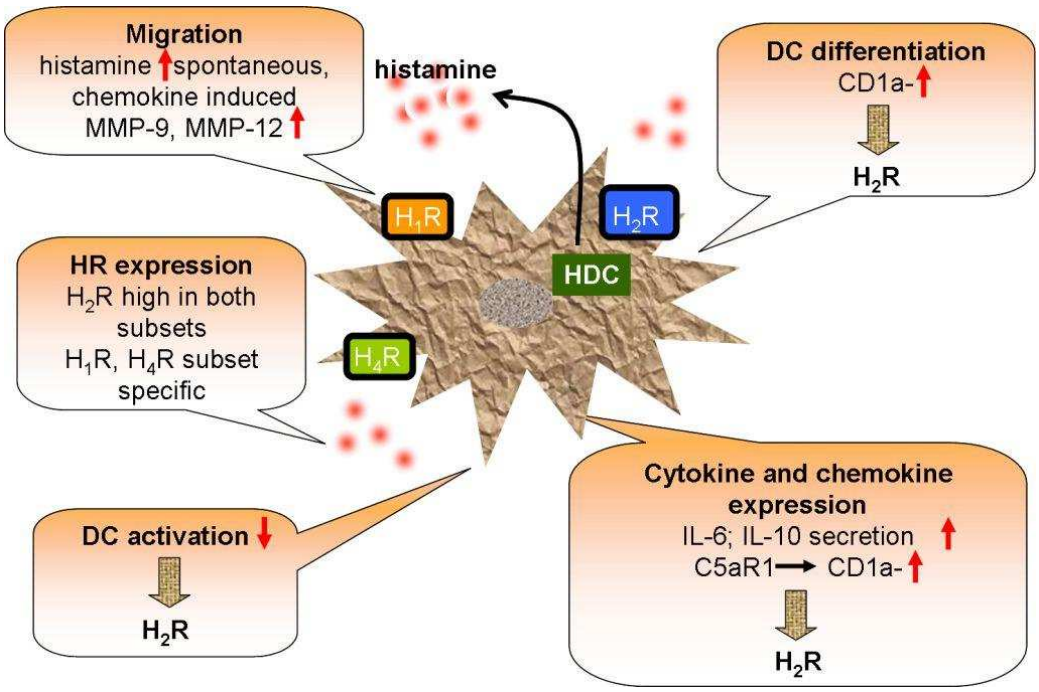

Figure 30.

Summary of the results observed in the human system

H_2R was highly expressed by both DC subsets, whereas H_1R and H_4R showed subset-specific expression. Histamine modulated DC functions at different levels. It supported the development of $CD1a^-$ DCs and modulated DC activation through its inhibitory effect on $CD1a^+$ DC differentiation. The histamine-induced reduction of $CD1a^+$ DCs resulted in increased secretion of IL-6 and IL-10. Histamine also induced DC migration of both subsets and modulated the production of MMP enzymes. Furthermore, it modulated the expression of C5aR1. All these effects were mediated through H_2R.

The *in vivo* and *in vitro* studies on the role of histamine on murine and human DCs revealed that histamine mediated several cellular activities of both murine and human DCs. The absence of H_4R resulted in an enhanced antigen presentation capability and adhesion characteristics of mouse DCs. Furthermore, H_4R deficiency decreased mouse DC migration and gene expression of some cytokines as well as it altered the inducibility of cytokine mRNA production. We have to take into consideration that changes observed here, are more significant in the case of chronic lack of H_4R rather than in the acute inhibition of the receptor. This suggests that defect of H_4R may also influence DC functions both in a direct and indirect manner. Furthermore, our results demonstrated that histamine had a profound effect on the development of human $CD1a^+$ DCs that was mediated by H_2R. This regulation had a further impact on cytokine production known to be different in the $CD1a^-$ and $CD1a^+$ subsets. As H_2R was expressed at high levels in both DC subsets, it may dominate the regulation of

multiple DC-related functional activities. In contrast, H_1R and H_4R with opposing subset-related expression may have a regulatory or fine-tuning role in histamine-induced functional activities.

Histamine is not only the major mediator of acute inflammatory and immediate hypersensitivity responses, but has also been demonstrated to affect chronic inflammation and several essential functions of the immune system. It is also well known that DCs, which control homeostatic processes and regulate acute and chronic inflammation can be affected by this small biogenic molecule. Thus the knowledge about the molecular mechanism of histamine actions on DCs has crucial importance to develop effective therapeutic strategies for the treatment of inflammatory diseases.

As DCs may localize to the vicinity of histamine producing cells, such as basophils and mast cells present in an inflammatory environment, they are potential targets of histamine. The results gained in our study highlighted some interesting aspects of histamine and DC crosstalk, which may help in better understanding the underlying mechanisms of histamine-mediated conditions. Indeed, more research is needed to understand the complex role of DCs in histamine-mediated immune responses, however by every step we are closer to utilize the experimental results in clinical settings especially in drug design and development.

8. SUMMARY

Dendritic cells (DCs) act as special sentinels of the immune system and form a functional bridge between innate and adaptive immune responses to maintain internal stability. During their life cycle DCs are continuously patrolling peripheral tissues and lymphoid organs and are affected by various stimuli that involve microbial insults or abnormal self structures.

In our studies we investigated the functional cross-talk of histamine and DCs in two experimental systems. The effects of histamine on various DC types were compared in a murine system by using genetically modified strains *in vivo* and by using mouse tissue-derived DCs *in vitro*. In humans we analysed the effects of histamine on the differentiation and functional activities of two previously identified DC subtypes.

First we demonstrated the expression of various histamine receptors (HRs) by mouse DCs at the protein level and showed that the expression of H_2R and H_4R was decreasing during *in vitro* bone marrow-derived DC differentiation, whereas that of the H_4R elevated significantly as a result of LPS stimulation. We also showed that histamine was able to reduce antigen presentation of splenic DCs significantly through H_4R without alterations in the expression of relevant cell surface molecules. Our migration studies revealed that neither histamine nor the H_4R agonist 4-MH influenced mouse DC migration significantly, however chronic histamine treatment in the course of DC differentiation stimulated cell migration in a time-dependent manner. It has also been proven that in the absence of H_4R DCs exhibited a significantly reduced migration capacity when compared to WT cells. Both transient and sustained presence of histamine increased adhesion of murine DCs via H_4R and mRNA expression of IL-1ß was increased by the combined effect of H_4R agonist and LPS treatment. This significant change could be reversed by the H_4R blocker JNJ7777120, underpinning the impact of histamine on DC cytokine expression via H_4R. Comparison of the cytokine profiles of WT and $H_4R^{-/-}$ DCs *in vivo* revealed significant alterations in the mRNA expression of Th1 and Th2 cytokines in $H_4R^{-/-}$ DCs as compared to WT cells.

In the second set of experiments we tested how histamine influences the development and functional activities of $CD1a^-$ and $CD1a^+$ DC subsets differentiated *in vitro* from human $CD14^+$ blood monocytes together with the HR dependence of these effects. We found that histamine modulated DC differentiation by supporting the development of $CD1a^-$ DCs. This effect was attributed to H_2R as only its specific synthetic inhibitor could restore the generation

of $CD1a^+$ cells. Testing HR expression of $CD1a^-$ and $CD1a^+$ cells we demonstrated that H_2R was highly expressed by both DC subsets, whereas H_1R and H_4R were expressed in a subset-specific manner. Histamine modulated DC activation through its inhibitory effect on $CD1a^+$ DC differentiation and the histamine-induced reduction of $CD1a^+$ DCs resulted in increased secretion of IL-6 and IL-10 cytokines. Histamine modulated the expression of C5aR1 in a H_2R and subset specific manner and induced spontaneous and chemokine-mediated DC migration of both subsets. Furthermore it modulated the production of the MMP-9 and MMP-12 enzymes also involved in the regulation of DC migration.

We hope that our results will contribute to a better understanding of the relationship between DCs and histamine.

9. ÖSSZEFOGLALÁS

Az „őrszemként” működő dendritikus sejtek (DS) funkcionális hidat képezve a természetes és az adaptív immunválasz folyamatai között nélkülözhetetlenek a szervezet belső egyensúlyának fenntartásában. A perifériás szövetek és a limfoid szervek között vándorolva életciklusuk során számos környezeti inger, így mikrobiális, metabolikus és más környezeti hatások érhetik őket, aminek eredményeként aktiválódnak és immunfolyamatok sorát indíthatják el.

Kísérleteink során kétféle kísérleti rendszerben vizsgáltuk a hisztamin DS-ekre kifejtett hatását. Egér rendszerben genetikailag módosított törzsek segítségével *in vivo* és szöveti DS-ekkel *in vitro* hasonlítottuk össze a hisztamin és receptorainak fontosságát különböző DS funkciókban. Emberi monocita eredetű DS-ek előzetesen azonosított altípusaiban a hisztamin sejt differenciációra és a DS funkciókra kifejtett hatását tanulmányoztuk.

Elsőként egér DS-ekben igazoltuk a különböző hisztamin receptorok fehérje szintű expresszióját és megállapítottuk, hogy a H_2R és H_4R expressziója csökken az *in vitro* DS differenciálódás során, míg a H_4R expressziója LPS aktiváció határára szignifikánsan nő. Igazoltuk, hogy a hisztamin a H_4R-on keresztül szignifikánsan csökkenti a lép DS-ek antigén prezentáló képességét anélkül, hogy eltérést tapasztaltunk volna a sejtfelszíni molekulák kifejeződésében. Migrációs vizsgálataink rámutattak, hogy sem a hisztamin, sem pedig a 4-MH (H_4R agonista) nem vált ki szignifikáns változást a DS-ek migrációjában, azonban a hosszan tartó hisztamin kezelés a DS-ek fejlődése során a kezelés időtartamával egyenes

arányban fokozza a sejtek migrációját. Azt is igazoltuk, hogy a H_4R hiányában fejlődő DS-ek szignifikánsan alacsonyabb vándorlási képességgel rendelkeznek, mint a vad típusú sejtek. A hisztamin a H_4R közvetítésével a hisztamin átmeneti vagy hosszantartó jelenlétében egyaránt növeli az egér DS-ek adhéziós képességét. Az LPS stimulációt követő emelkedett IL-1ß expressziót a H_4R agonista (4-MH) kezelés tovább emeli, míg specifikus antagonista (JNJ7777120) alkalmazásával ez a hatás megszűnt, igazolva a hisztamin H_4R-on át zajló citokin termelődést befolyásoló hatását. A vad típusú és $H_4R^{-/-}$ DS-ek Th1 és Th2 citokin profilját *in vivo* rendszerben hasonlítottuk össze és szignifikáns változásokat tapasztaltunk a $H_4R^{-/-}$ és a vad típusú DS-ek mRNS kifejeződésében.

In vitro körülmények között emberi monocitából differenciáltatott $CD1a^-$ és $CD1a^+$ DS alpopulációkban vizsgáltuk a hisztamin differenciációra és DS funkciókra kifejtett hatását és összefüggését a hisztamin receptorok (HR) típusával.

Eredményeink azt igazolják, hogy a hisztamin szabályozza a DS altípusok differenciálódását, elősegítve a $CD1a^-$ sejtek fejlődését. Ezt a hatást a H_2R-nak tulajdonítjuk, mivel csak ezen HR specifikus inhibitora tudja visszaállítani a $CD1a^+$ sejtek képződését. Kimutattuk továbbá, hogy a H_2R magasan expresszálódik mindkét DS altípusban, míg a H_1R és H_4R DS altípus-specifikus kifejeződést mutat. A hisztamin a $CD1a^+$ DS-ek differenciálódására kifejtett gátló hatásán keresztül szabályozza a DS-ek aktivációját, fokozva a $CD83^+CD1a^-$ sejtek képződését, miközben a hisztamin által kiváltott $CD1a^+$ sejtszám csökkenés eredményeképpen nő az IL-6 és IL-10 citokinek szekréciója. A hisztamin a H_2R-on keresztül, altípus specifikus módon szabályozza bizonyos migrációhoz kapcsolódó gének (C5aR1, CXCR4, CX3CR1) expresszióját, mindkét altípusban fokozza a DS-ek spontán és kemokin közvetített migrációját és szabályozza az MMP-9 és MMP-12 enzimek termelődését, melyek szintén szerepet játszanak a DS-ek vándorlásának irányításában.

Reméljük, hogy eredményeink hozzájárulnak a DS-ek és a hisztamin kapcsolatának jobb megértéséhez.

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11. PUBLICATIONS

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DEBRECENI EGYETEM EGYETEMI ÉS NEMZETI KÖNYVTÁR
KENÉZY ÉLETTUDOMÁNYI KÖNYVTÁRA

Iktatószám: DEENKÉTK /12/2012.
Tételszám:
Tárgy: Ph.D. publikációs lista

Candidate: Tünde Simon

Neptun ID: UIF8DA

Doctoral School: Doctoral School of Molecular Cell and Immune Biology

List of publications related to the dissertation

1. **Simon, T.**, Gogolák, P., Kis-Tóth, K., Jelinek, I., László, V., Rajnavölgyi, É.: Histamine modulates multiple functional activities of monocyte-derived dendritic cell subsets via histamine receptor 2. *Int. Immunol. Epub ahead of print (2012)*
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2. **Simon, T.**, László, V., Falus, A.: Impact of histamine on dendritic cell functions. *Cell Biol. Int. 35* (10), 997-1000, 2011.
   DOI: http://dx.doi.org/1042/CBI20100844
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3. **Simon, T.**, László, V., Lang, O., Buzás, E., Falus, A.: Histamine regulates relevant murine dendritic cell functions via H4 receptor. *Front Biosci (Elite Ed). 3*, 1414-1424, 2011. *

4. **Simon, T.**, Jelinek, I., Apponyi, G., László, V., Rajnavölgyi, É., Falus, A.: Expression and function of histamine H4 receptor in mouse splenic dendritic cells. *Inflamm. Res. 59* (Suppl.2), S201-S203, 2010.
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List of other publications

5. **Simon, T.**, F. Semsei, Á., Ungvári, I., Hadadi, É., Virág, V., Nagy, A., S. Vángor, M., László, V., Szalai, C., Falus, A.: Asthma endophenotypes and polymorphisms in the histamine receptor HRH4 gene.
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PLoS One. 6 (8), e23653, 2011.
DOI: http://dx.doi.org/10.1371/journal.pone.0023653
IF:4.411 (2010)

Total IF: 13.698

Total IF (publications related to the dissertation): 7.052

** In the editorial invitation received in 2009 the editor of Frontiers in Bioscience clearly referred to the impact factor of the journal (IF: 3.6 in 2008), however next year the journal divided into 3 sections and at calculation of IF in 2010 the new "Landmark edition" has already published less articles (IF: 4.048). Thus, regarding the article published in "Elite edition" no IF is available. The publication is included in the PubMed and Scopus, its type is "peer-reviewed original article".*

The Candidate's publication data submitted to the Publication Database of the University of Debrecen have been validated by Kenezy Life Sciences Library on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

13 February, 2012

DEBRECENI EGYETEM KENÉZY ÉLETTUDOMÁNYI KÖNYVTÁRA EGYETEMI ÉS NEMZETI KÖNYVTÁR

Posters and presentations at conferences:

Detection and function of H4R in spleen-derived dendritic cells
(European Histamine Research Society Conference, Fulda, 2009., poster presentation)

Detection and function of H4R in spleen-derived dendritic cells
(Semmelweis University PhD Days Conference, 2009., poster presentation)

Detection and function of H4R in spleen-derived dendritic cells
(Hungarian Society For Immunology Conference, Harkány, 2009., poster presentation)

Role of histamine and its H4 receptor in murine dendritic cell functions
(European Histamine Research Society Conference, Durham, 2010., oral presentation)

A hisztamin és a hisztamin H4 receptor szerepe az egér dendritikus sejtek működésében
(Hungarian Society For Immunology Conference, Szeged, 2010., oral presentation)

A hisztamin és a hisztamin H4 receptor szerepe az egér dendritikus sejtek működésében
(Semmelweis University PhD Days Conference, 2011., oral presentation)

Histamine modulates multiple functional activities of monocyte-derived dendritic cell subsets via histamine receptor 2
(World Immune Regulation Meeting VI. Conference, Davos, 2012., poster presentation)

12. KEY WORDS

histamine, histamine receptors, dendritic cells, antigen presentation, cell adhesion, cytokine, migration, CD1a molecule, dendritic cell activation

TÁRGYSZAVAK

hisztamin, hisztamin receptorok, dendritikus sejt, antigén prezentáció, sejt adhézió, citokin, migráció, CD1a molekula, dendritikus sejt aktiváció

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