

Histamine down-regulates IL-27 production in antigen-presenting cells

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ABSTRACT

Histamine is a potent mediator in allergic inflammation with immunomodulatory properties. Since histamine was described to inhibit IL-12 production in human APCs, we hypothesized that also the expression of IL-27, a newly described member of the IL-12 family, which is present in inflammatory skin lesions, is modulated by histamine. Stimulation of human monocytes with histamine resulted in significant reduction of TLR ligand-induced IL-27 production in human monocytes. IL-27 subunits, p28 and EBI3, were down-regulated at the mRNA and protein level, whereas other cytokines, such as IL-6, IL-10, and TNF- α , were not influenced. Studies with histamine receptor-specific agonists and antagonists showed that the down-regulation of IL-27 was mediated via H₂R and H₄R but not H₁R and H₃R. Human KCs treated with supernatants of histamine-prestimulated monocytes induced significantly less CXCL10 than supernatants containing high levels of IL-27. DCs from H₄R^{-/-} mice responded to TLR stimulation with higher IL-27 production as compared with WT mice. The down-regulation of IL-27 by histamine might be a new mechanism in the pathogenesis of inflammatory skin diseases, in particular, if increased concentrations of histamine are present at sites of inflammation, such as in chronic eczema and psoriasis. *J. Leukoc. Biol.* 92: 000–000; 2012.

Introduction

Histamine is one of the most important immunomodulatory mediators in allergic and inflammatory diseases. Histamine can bind to four different seven-transmembrane receptors, signaling via coupled G-proteins. Histamine receptors are ex-

pressed in different tissues and play diverse functions in allergic inflammatory responses (H₁R, H₂R, H₄R) [1,2] in the regulation of gastric acid secretion (H₂R) and in the nervous system (H₃R). The latest discovered H₄R seems to play an important role in immune responses, as its expression was shown on many cell types involved in inflammatory and allergic diseases, such as mast cells, DCs, eosinophils, and T cells. The stimulation of the H₄R on mast cells, MoDCs, and eosinophils leads to chemotaxis [3–5], on CD8⁺ T cells the chemoattractant IL-16 was shown to be up-regulated after H₄R stimulation [6], and also, Th2 cells were recently shown to functionally express the H₄R [7]. As in MoDCs, monocyte-derived inflammatory epidermal DCs, and Langerhans cells, H₄R-dependent suppression of IL-12 production was described [4, 8, 9], we hypothesized that also IL-27, a novel member of the IL-12 cytokine family, can be influenced by H₄R stimulation.

IL-27 is a heterodimeric cytokine composed of the two subunits, EBI3 and p28. EBI3 is a homologue to IL-12p40, which was identified by Devergne et al. [10] and shown to couple with p35 to form the cytokine IL-35 [11]. However, no biological function of EBI3 was described until Pflanz et al. [12] computationally identified p28 and showed its pairing with EBI3 to form IL-27, which was described to be an early product of human-activated monocytes and murine macrophages [12], and its expression can be induced by inflammatory trigger factors, such as LPS and CD40 ligation [12, 13]. IL-27R is composed of the widely used signal transduction subunit gp130, coupled to the cytokine receptor WSX-1 (or alternatively named T cell cytokine receptor) [14]. The IL-27R is expressed on various cells of the innate and adaptive immune system, including epithelial cells, DCs, monocytes, NK cells, and T cells [14]. Regarding its function, it was shown that IL-27 induces proliferation in human as well as murine naïve T cells but not in memory T cells [8]. Moreover, IL-27 induced IFN- γ production in human T cells in synergy with IL-12, thereby promoting Th1 responses [12]. Mouse studies confirm the role of IL-27 in the

Abbreviations: ^{-/-}=deficient, BMDC=bone marrow-derived DC, EBI3=EBV-induced gene 3, hr=human recombinant, H_xR=histamine receptor, KC=keratinocyte, LTA=lipoteichoic acid, MoDC=monocyte-derived DC, poly I:C=polyinosinic:polycytidylic acid

The online version of this paper, found at www.jleukbio.org, includes supplemental information.

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regulation of early Th1 responses, as *WSX-1*^{-/-} mice showed impaired Th1 responses in early-phase immune responses and were shown to have higher susceptibility to intracellular pathogens such as *Leishmania* and *Listeria* [15, 16]. As monocytes themselves express the IL-27R, autocrine effects on the production of IL-1 β , TNF- α , IL-12, and IL-18, mediated via the phosphorylation of Stat1 and Stat3, have been observed [14]. Recently, we and others [17, 18] showed that KCs also express a functional IL-27R and respond to IL-27 stimulation with the up-regulation of chemokine secretion and increased surface expression of MHC-II, CD54, and IL-18R α . Moreover, increased levels of IL-27 were detected in chronic eczematous lesions [17] and in the dermis of psoriasis patients [18].

As the immunomodulatory mediator histamine is also present in chronic lesions of inflammatory skin diseases, such as psoriasis [19] and atopic eczema [20], there might be a link between histamine and IL-27. Therefore, we intended to investigate the influence of histamine on IL-27 production in human monocytes and murine BMDCs and functional effects thereof.

MATERIALS AND METHODS

Reagents

Various histamine receptor ligands were used in this study: histamine (agonist for all histamine receptors; Alk-Schering, Wedel, Germany), 2-pyridylethylamine (selective H₁R agonist; Tocris Bioscience, Bristol, UK), amthamine (highly selective H₂R agonist; Tocris Bioscience), (*R*)-(-)- α -methylhistamine (selective H₃R agonist; Tocris Bioscience), 4-methylhistamine (H₄R agonist; Tocris Bioscience), clemastine (selective H₁R antagonist; Novartis, Nürnberg, Germany), ranitidine (selective H₂R antagonist; Biomol, Hamburg, Germany), ciproxifan (selective H₃R inverse agonist; Sigma-Aldrich, Deisenhofen, Germany), and JNJ7777120 (selective H₄R antagonist, also shows low activity on H₂R; Sigma-Aldrich). If not stated differently, histamine receptor ligands were used at a concentration of 10 μ M.

Other reagents for stimulation of human monocytes were used as follows: hrIFN- γ (200 ng/ml; Peprotech Hamburg, Germany), hrIL-27 (100–500 pg/ml; R&D Systems, Wiesbaden, Germany), LPS (50 ng/ml; Sigma-Aldrich), poly I:C (10 μ g/ml; Sigma-Aldrich), and hrTNF- α (10 ng/ml; Peprotech). For blocking/neutralization of IL-27, we used 1 μ g/ml of a polyclonal antibody provided by R&D Systems.

For the stimulation of murine BMDCs, histamine (0.5 mM; Alk-Schering), LPS (1 μ g/ml, from *Escherichia coli* 0111:B4; Sigma-Aldrich), LTA (10 μ g/ml, isolated from *Staphylococcus aureus*, as described previously [21]; University of Konstanz, Germany), and PGN (10 μ g/ml; Sigma-Aldrich) were used.

Isolation, culture, and stimulation of human monocytes

PBMCs were separated from buffy coats by density centrifugation on lymphoprep (Fresenius Kabi Norge AS, Oslo, Norway), and erythrocytes were removed by incubation with Gey's lysis buffer. Monocytes were isolated using magnetic beads (Miltenyi Biotec, Bergisch-Gladbach, Germany). The isolated monocytes had a purity of at least 95%, as evaluated by flow cytometry, and included minimal numbers of B lymphocytes (CD19, <0.5%), T lymphocytes (CD3, <0.5%), and NK cells (CD56, <3%), and granulocytes (CD16) were absent.

Monocytes were cultured in RPMI 1640, supplemented with 2 mM L-glutamine, 100 mg/ml penicillin/streptomycin, 12 mM HEPES, and 5% v/v FCS (PAN-Biotech, Aidenbach, Germany; all other media components from Biochrom, Berlin, Germany) at 37°C in a humidified atmosphere containing 5% CO₂.

Monocytes were stimulated with 10 μ M histamine receptor agonists for 24 h, and then the cells were activated by addition of poly I:C or IFN- γ and

LPS (time gap of 2 h during stimulation). RNA was lysed after 4, 8, and 24 h, and supernatants were taken after 24 h. For blocking experiments, cells were treated with receptor antagonists, 30 min before the stimulation with histamine receptor agonists.

Isolation, culture, and stimulation of human KCs

Human primary KC cultures were prepared from foreskin, which was cut into pieces and incubated overnight at 4°C in 2.4 U dispase II (Roche Molecular Biochemicals, Mannheim, Germany). The next day, the epidermis was separated from the dermis and placed for 20 min at 37°C in EDTA (0.02%)–trypsin (0.05%) solution (PAN-Biotech). After stopping the trypsin reaction by addition of FCS (PromoCell, Heidelberg, Germany), the cell suspension was filtered through a sterile gauze (40 μ m) and washed two times in PBS. The obtained single-cell suspension of KC was incubated in serum-free growth medium, Keratinocyte Growth Medium 2 Kit (PromoCell), at 37°C in a humidified atmosphere containing 5% CO₂. For experiments, KC, in Passages 3–6, were cultured in hydrocortisone and epidermal growth factor-free medium until they reached 80% confluence.

The supernatants used for the stimulation of KC were obtained as follows: monocytes were stimulated with 10 μ M histamine for 24 h, and then the cells were activated by addition of IFN- γ and LPS (time gap of 2 h during stimulation); 24 h later, the cells were washed intensively to remove IFN- γ , which might directly stimulate KC. The presence of residual IFN- γ was determined at the end of the experiment by ELISA; compared with the initial stimulation (200 ng/ml), the concentration of IFN- γ after washing was 0.1–0.5 ng/ml, in histamine-stimulated and nontreated conditions (data not shown).

The monocytes were incubated with fresh supplemented RPMI 1640, and 24 h later, supernatants were taken. The monocyte supernatants were used for the stimulation of KC together with TNF- α (10 ng/ml), which was applied 2 h before the stimulation with supernatants. Control cells (no addition of monocyte supernatant) were cultured in supplemented RPMI 1640 or treated with TNF- α . For rescue experiments, hrIL-27 was added to the KC culture (ranging from 100 to 500 pg/ml, depending on the histamine-induced reduction of IL-27 measured by ELISA). KC supernatants were taken after 24 h of stimulation, and the production of CXCL10 was measured. A schematic presentation of the experimental setup is depicted below (see Fig. 7A).

Mice

BALB/c mice were obtained from Charles River (Sulzfeld, Germany) at the age of 6–8 weeks. The H₄R^{-/-} mice were generated by Lexicon Genetics (Woodlands, TX, USA), as described previously [3]. Mice were bred in the animal facility of the University of Veterinary Medicine Hannover Foundation (Germany). All animals were healthy and were housed in groups of six mice/cage at 22°C, with a 12-h light/dark cycle. Water and standard diet (Altromin, Lage/Lippe, Germany) were available ad libitum.

Isolation, culture, and stimulation of murine BMDCs

Murine BMDCs were generated as described previously [22]. Briefly, BMDCs were cultured in RPMI 1640 (Biochrom), supplemented with 10% FCS (Biochrom), 50 μ mol/l β -ME (Sigma-Aldrich), and 20 ng/ml GM-CSF (R&D Systems). Fresh medium supplemented with GM-CSF was added on Days 3, 6, and 8. FACS analysis of the Day 10 cell suspension demonstrated a high yield of CD11c and MHC class II-positive cells. BMDCs from WT and H₄R^{-/-} mice were stimulated at Day 9 of culture with LPS, LTA, PGN, or histamine for 24 h.

mRNA isolation, reverse transcription, and quantitative RT-PCR

Isolated monocytes (2 $\times$ 10⁵) were stimulated in supplemented RPMI 1640 for the indicated time-points. Monocytes were washed in PBS and lysed for RNA isolation using Mini RNA Isolation II Kit (Zymo Research, Orange, CA, USA), and reverse transcription was done with the First Strand cDNA

Synthesis Kit (MBI Fermentas, St. Leon-Rot, Germany). Real-time quantitative PCR was performed on a LightCycler (Roche Molecular Biochemicals) using SYBR Green with Quantitect primer assays for GAPDH (QT01192646), EBI3 (QT01014104), IL-27p28 (QT00236250), IL-6 (QT00083720), TNF- α (QT01079561), and IL-10 (QT00041685), according to the manufacturer's instructions (Qiagen, Hilden, Germany). The following PCR settings were used: an initial activation step of 15 min at 95°C with ramp 20°Cs⁻¹ was followed by three-step cycling (45 cycles)—denaturation 15 s, 94°C; annealing 20 s, 55°C; extension 20 s, 72°C (all three with ramp 2°Cs⁻¹). Melting curve analysis was performed from 60°C to 90°C with ramp 20°Cs⁻¹. The amount of target genes relative to the reference GAPDH was quantified using the Relative Quantification Software (Roche Molecular Biochemicals).

ELISA

Cell-free supernatants were used to detect the human cytokines IL-27, CXCL10, IFN- γ , IL-6, IL-10, and TNF- α in ELISA, performed according to the manufacturer's instructions (R&D Systems and eBioscience, San Diego, CA, USA, respectively). Murine cytokines IL-27, IL-6, IL-10, and TNF- α were measured in ELISA obtained from R&D Systems.

Western blot

Isolated monocytes (1×10^6) were stimulated with histamine in supplemented RPMI 1640; 24 h later, cells were activated by addition of IFN- γ and 10 min later, LPS. Fifteen and 45 min after LPS stimulation, the cells were homogenized in M-Per mammalian extraction reagent (Thermo Fisher Scientific, Ulm, Germany). After cell lysis, the samples were centrifuged at 4°C, 3000 g for 10 min, and the protein concentration in the supernatants was determined with a Lowry protein assay (Bio-Rad, Munich, Germany). A total of 25 μ g protein/sample was separated on 5–16% SDS-polyacrylamide gels (Pierce, Rockford, IL, USA) under reducing conditions and transferred via semidry blotting (30 min, 20 V) onto a 0.45- μ m nitrocellulose membrane (Roth, Karlsruhe, Germany). Antibodies obtained from Cell Signaling Technology (distributed via New England Biolabs,

Frankfurt, Germany) were used to detect β -actin, phospho-Stat1, Stat1, phospho-p44/42 MAPK (phospho-Erk1/2), p44/32 MAPK (Erk1/2), and phospho-NF- κ B p65, followed by a HRP-conjugated horse anti-mouse or horse anti-rabbit secondary antibody. Immunoreactivity was detected using a chemiluminescence kit, according to the instructions of the manufacturer (Pierce). Densitometric analysis of the blots was performed using the program ChemiImager (Alpha Innotech, San Leandro, CA, USA).

Statistical analysis

Before statistical analysis, a normality test was performed; for calculation of *P* values, either a paired *t* test (see Figs. 1–5 and 7) or unpaired *t* test (see Fig. 6) was used; a *P* value <0.05 was regarded as significant. *P* < 0.05 is depicted with *, *P* < 0.005 with **, and *P* < 0.001 with ***. The program GraphPad Prism version 5 (GraphPad Software, La Jolla, CA, USA) was used for statistical analysis.

Ethics

The investigation of the role of histamine receptors in allergic skin inflammation was approved by the local ethics committee of Hannover Medical School (Vote Nr. 4253) and was conducted according to the Declaration of Helsinki Principles.

RESULTS

Histamine down-regulates IL-27 mRNA and protein production in human monocytes

Human monocytes, which were prestimulated with 10 μ M histamine, showed reduced TLR-induced mRNA production of the two subunits of IL-27, p28 and EBI3. The down-regulation was highly pronounced after 4 h of stimulation; at later time-points—8 h and 24 h of stimulation—the effect was observed

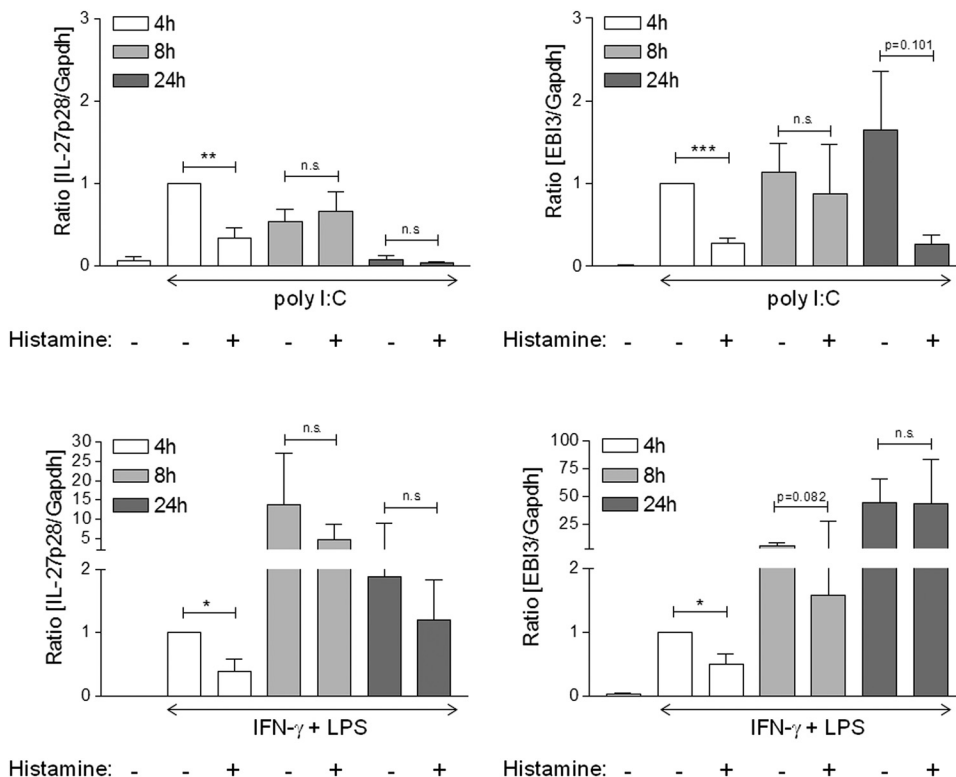


Figure 1. Histamine stimulation reduces the mRNA production of IL-27 subunits p28 and EBI3. Monocytes were stimulated for 24 h with histamine and were then activated with poly I:C or IFN- γ and LPS for 4 h (white bars), 8 h (light gray bars), or 24 h (dark gray bars). mRNA levels were measured by real-time quantitative LightCycler PCR. Mean and SEM of seven independent experiments are shown.

less clearly (Fig. 1). Histamine reduced IL-27 mRNA production, independent of the TLR ligand used for the activation of monocytes, poly I:C (TLR3), or the combination of IFN- γ and LPS (TLR4), administered with a time gap of 2 h. Both stimulation protocols were shown previously to be effective in the induction of IL-12 family cytokines [4, 23]. In contrast to IL-27, the mRNA production of IL-6, TNF- α , and IL-10 was not changed significantly by histamine after 4 h of stimulation (the optimal time-point for blockade of IL-27; Fig. 2).

On the protein level, we observed differences in TLR ligand-induced cytokine production. The combination of IFN- γ and LPS was a more efficient inducer of IL-27, TNF- α , and IL-6 secretion, whereas poly I:C mainly induced the production of IL-10. Prestimulation with histamine resulted in decreased IL-27 production after TLR activation. For other cytokines, i.e., TNF- α , IL-6, and IL-10, no significant effect of histamine was detected (Fig. 3).

Histamine stimulation does not modulate Stat1, Erk1/2, and NF- κ B signaling

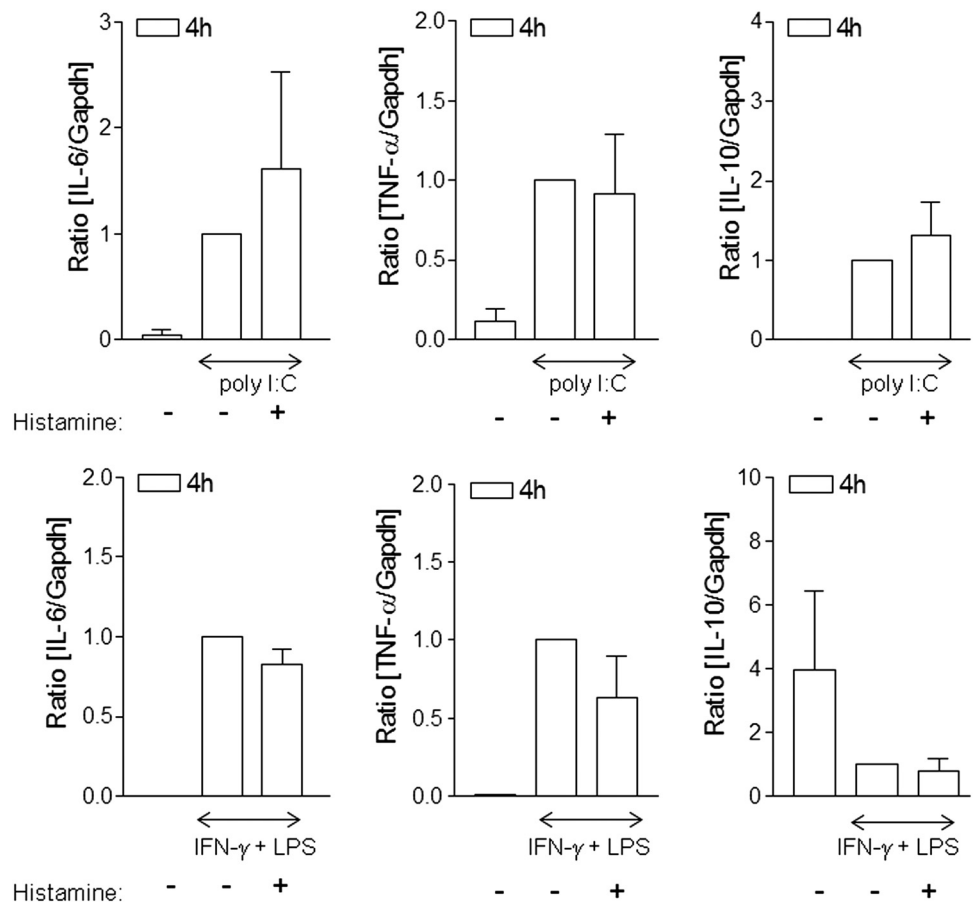
Concerning the involved signaling pathways, we investigated the effect of histamine on the phosphorylation of Stat1, Erk1/2, and NF- κ B. These signaling mediators are of relevance, as Stat1 and Erk1/2 were described to be regulated by histamine in other cell types [24, 25], and NF- κ B is involved in the induction of IL-27 production [13, 26]. Although only very

low amounts of phosphorylated Stat1, Erk1/2, and NF- κ B were detected in nonstimulated monocytes, activation with IFN- γ and LPS led to highly significant induction of phosphorylation. In monocytes prestimulated with histamine, we did not observe differences in any of the investigated signaling molecules. (Fig. 4).

The down-regulation of IL-27 protein in human monocytes is mediated via H₂R and H₄R

To evaluate which of the four histamine receptors is mediating the down-regulation of IFN- γ - and LPS-induced IL-27, we incubated human monocytes with specific receptor agonists and antagonists. We detected reduced IL-27 production after stimulation with the H₂R agonist anthamine and the H₄R agonist 4-methylhistamine, whereas the H₁R agonist 2-pyridylethylamine and the H₃R agonist (*R*)-(-)- α -methylhistamine had no effect. Preincubation of monocytes with the H₂R antagonist ranitidine and the H₄R antagonist JNJ777120 abolished IL-27 down-regulation by the corresponding agonists, confirming that in human monocytes, the effect is mediated via the H₂R and the H₄R (Fig. 5A). When we used histamine as a down-regulator of IL-27, we observed that H₂R antagonist alone and the combination of H₂R and H₄R antagonists abolished the histamine effect, whereas the H₄R antagonist alone was not sufficient (Fig. 5B). To further confirm that the dose of histamine ligands was optimal, we performed titration studies,

Figure 2. Histamine stimulation does not affect the mRNA production of IL-6, IL-10, and TNF- α . Monocytes were stimulated for 24 h with histamine and were then activated with poly I:C or IFN- γ and LPS for 4 h. mRNA levels were measured by real-time quantitative LightCycler PCR. Mean and SEM of seven (poly I:C) and five (IFN- γ and LPS) independent experiments are shown.



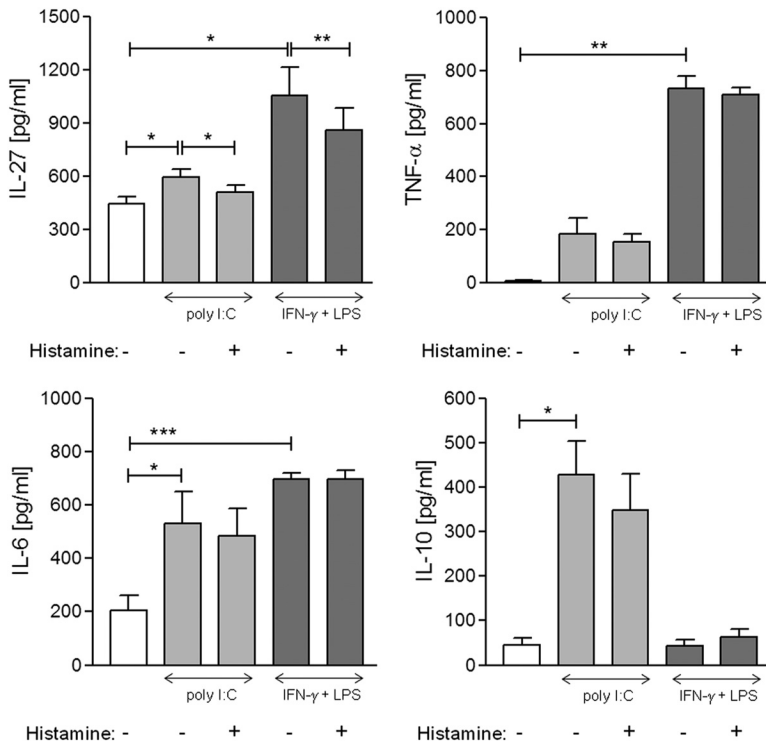


Figure 3. Histamine stimulation decreases IL-27 secretion into monocyte culture supernatants. Monocytes were stimulated for 24 h with histamine, followed by activation with poly I:C (light gray bars) or IFN-γ and LPS (dark gray bars) for 24 h, and determination of the cytokine content in the cell supernatant was done by ELISA. Histamine decreased IFN-γ- and LPS-induced IL-27 secretion but had no effect on IL-6, IL-10, and TNF-α (mean and SEM of at least six experiments are shown).

showing that the effect on IL-27 is dose-dependent, and the chosen dose was in the optimal range (Fig. 5C).

BMDCs from $H_4R^{-/-}$ mice produce higher amounts of IL-27 in the absence and presence of histamine

The secretion of IL-27 was investigated in murine BMDCs from BALB/c WT and $H_4R^{-/-}$ mice after stimulation with the

TLR ligands LPS, LTA, and PGN. All three agents induced higher IL-27 production in BMDCs from $H_4R^{-/-}$ mice as compared with WT BALB/c mice, whereas for IL-6, IL-10, and TNF-α production, no differences were observed (Fig. 6A). When BMDCs were treated with histamine, we observed down-regulation of LPS-induced IL-27 in $H_4R^{-/-}$ mice and WT mice (Fig. 6B), indicating that not only the H_4R but also an-

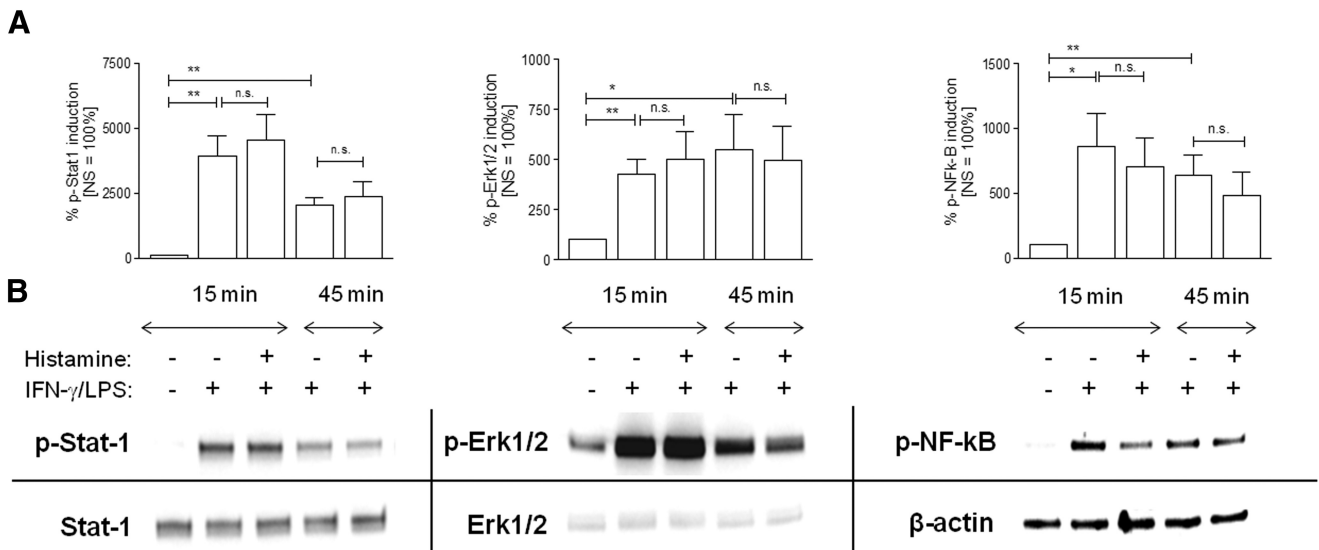


Figure 4. Histamine does not modulate Stat1, Erk1/2, and NF-κB phosphorylation in human monocytes. Monocytes were stimulated for 24 h with histamine and were then activated with IFN-γ and LPS for 15 min or 45 min. Western blot analysis showed no significant effect on the phosphorylation (p-) of Stat1, Erk1/2, and NF-κB phosphorylation. A summary (mean and SEM of eight, eight, and 16 experiments are depicted for Stat1, Erk1/2, and NF-κB, respectively) and representative experiments are shown in B.

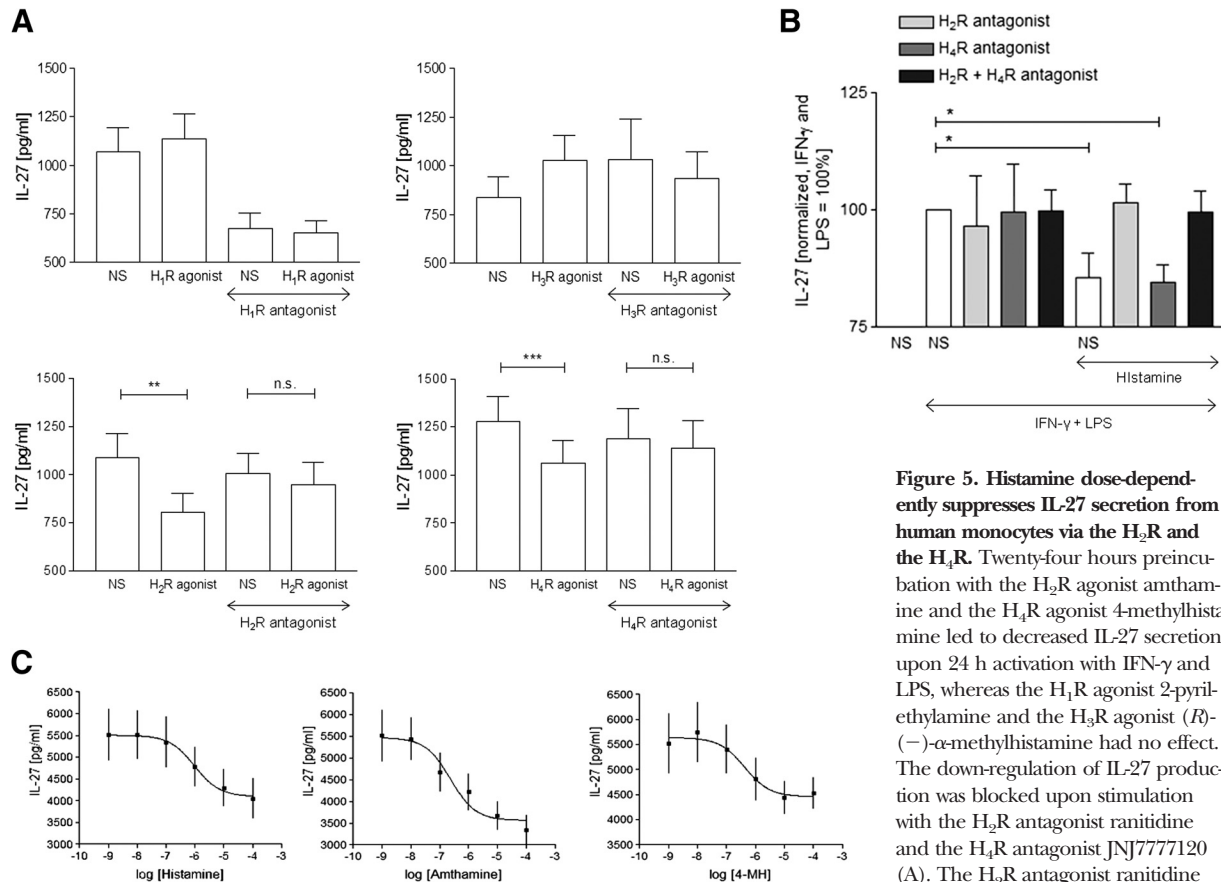


Figure 5. Histamine dose-dependently suppresses IL-27 secretion from human monocytes via the H₂R and the H₄R. Twenty-four hours preincubation with the H₂R agonist amthamine and the H₄R agonist 4-methylhistamine led to decreased IL-27 secretion upon 24 h activation with IFN- γ and LPS, whereas the H₁R agonist 2-pyril-ethylamine and the H₃R agonist (*R*)-(-)- α -methylhistamine had no effect. The down-regulation of IL-27 production was blocked upon stimulation with the H₂R antagonist ranitidine and the H₄R antagonist JNJ7777120 (A). The H₂R antagonist ranitidine and the combination of H₂R antago-

nist with the H₄R antagonist JNJ7777120 blocked histamine-induced IL-27 down-regulation (B). The reduction in IL-27 secretion was dose-dependently induced by histamine, the H₂R agonist amthamine, and the H₄R agonist 4-methylhistamine ([4-MH]; C). Mean and SEM of six, seven, and eight independent experiments are shown in A, B, and C, respectively.

other histamine receptor, presumably the H₂R, plays a role in the regulation of IL-27.

KCs cultured in histamine-treated monocyte supernatants produce less CXCL10

We described recently that KCs respond to TNF- α and subsequent IL-27 stimulation (2 h later) with high production of the chemokine CXCL10 (also known as IFN-inducible protein 10) [17]. In accordance with these findings, we observed high CXCL10 production in KCs incubated with IFN- γ and LPS-stimulated monocyte supernatants, which show high IL-27 content (Fig. 7B and C). The level of CXCL10 was reduced significantly when monocyte supernatants pre-stimulated with histamine were used for the culture of KCs (Fig. 7C). The addition of hrIL-27 to the supernatants restored CXCL10 production (Fig. 7D). Blocking of IL-27 in monocytes in the presence of IFN- γ and LPS resulted in similar down-regulation of CXCL10, as observed after histamine treatment. The combined treatment with histamine and IL-27 neutralizing antibody did not further decrease CXCL10 expression in KCs (Fig. 7E).

DISCUSSION

Several studies have shown a role of histamine in the regulation of DC and monocyte function, mainly regarding to chemotaxis and the

release of inflammatory mediators, such as IL-12 and CCL2 [4, 8]. As a result of these findings, we hypothesized that histamine might influence the production of IL-27, a novel member of the IL-12 family of cytokines, and immunological responses resulting from IL-27R activation on immune cells. It was already known from previous studies that monocytes, as the main producers of IL-27, express histamine receptors and that IL-12 secretion is impaired upon stimulation with histamine [4]. We induced IL-27 production in monocytes by treatment with poly I:C or by the combined treatment with LPS and IFN- γ with mRNA and protein induction patterns, similar to studies on IL-27 performed previously [12, 13]. When we added histamine to our monocyte cultures, we observed a decrease in IL-27 production on the mRNA and protein level. This effect appears to be fairly selective, as other cytokines, such as TNF- α , IL-6, and IL-10, were not down-regulated after histamine stimulation. Although the investigated cytokines share the NF- κ B pathway as inducer of their production [27], there exist alternative ways to regulate, e.g., IL-10 via Erk1/2 and p38 [28], IL-6 via Jak/Stat [29], and TNF- α via MEK and Erk1/2 [30]. These findings indicate that differentially used signaling pathways could be the reason for the selective action of histamine on cytokines of the IL-12 family, whereas other cytokines are not affected.

Concerning the involved signaling pathways, we investigated NF- κ B, as the transcription of both subunits of IL-27 was shown

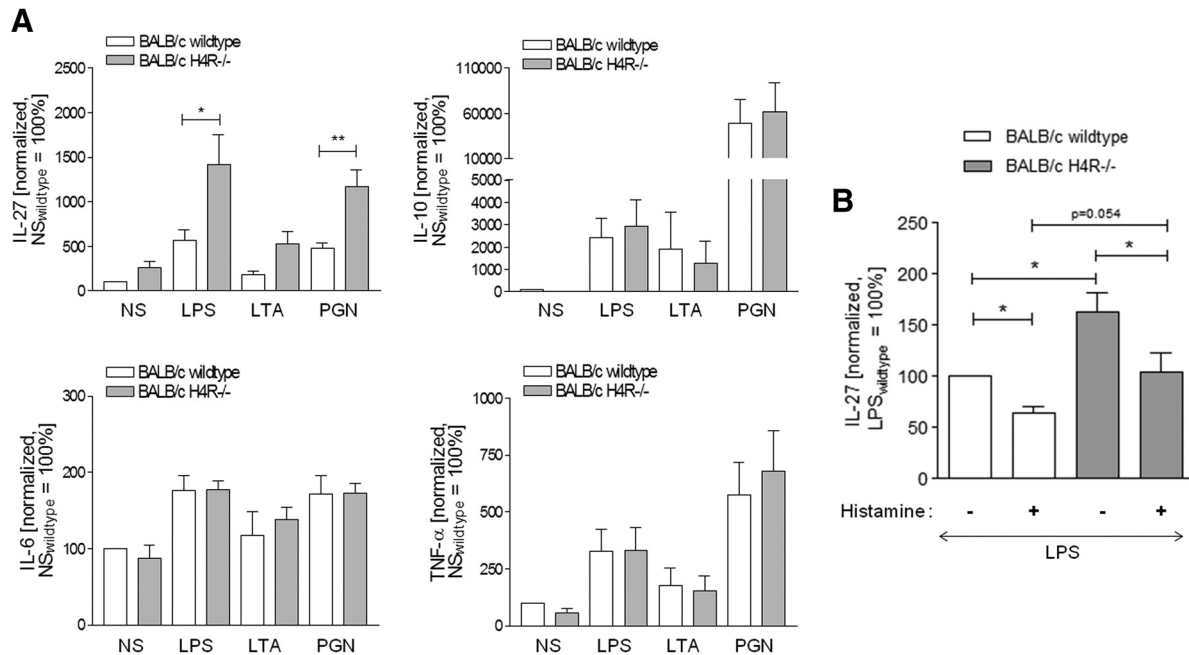


Figure 6. BMDCs from $H_4R^{-/-}$ mice produce higher amounts of IL-27. BMDCs were stimulated with LPS, LTA, and PGN for 24 h, and cytokines were measured by ELISA. (A) DCs derived from $H_4R^{-/-}$ mice (gray bars) secrete higher amounts of IL-27 than WT mice (white bars), whereas the secretion of IL-6, IL-10, and TNF- α is not modulated (mean and SEM of at least five experiments are shown; basal cytokine expression ranged from 7 to 29 pg/ml for IL-27, from 0 to 157 pg/ml for IL-10, from 280 to 2373 pg/ml for IL-6, and from 48 to 570 pg/ml for TNF- α). (B) BMDCs from $H_4R^{-/-}$ mice and WT mice respond to histamine treatment (0.5 mM) with down-regulation of IL-27 (mean and SEM of four independent experiments are shown).

to be induced by LPS signaling via TLR4 and NF- κ B [13, 26]. In addition, we investigated effects of histamine on Stat1 and Erk1/2 phosphorylation, as histamine was described previously to influence these signaling molecules [24, 25]. However, we did not observe differences in the phosphorylation status of Stat1, Erk1/2, and NF- κ B in our experiments. The study showing that histamine down-regulates Stat1 used a stimulation time of 48 h before measurement of Stat1 [24], which is much longer compared with the short time effects that were measured in our study. Morse et al. [25] showed Erk1/2 regulation via histamine in human embryonic kidney cells; however, another study performed in our group [4] could not show Erk1/2 modulation in MoDCs. Therefore, it seems likely that the Erk1/2 pathway is of minor relevance in APCs but might be of note in other cell types. Thus, our studies could not reveal a signaling mechanism used by histamine to reduce IL-27 production in monocytes. This needs to be elucidated in more detail in further studies.

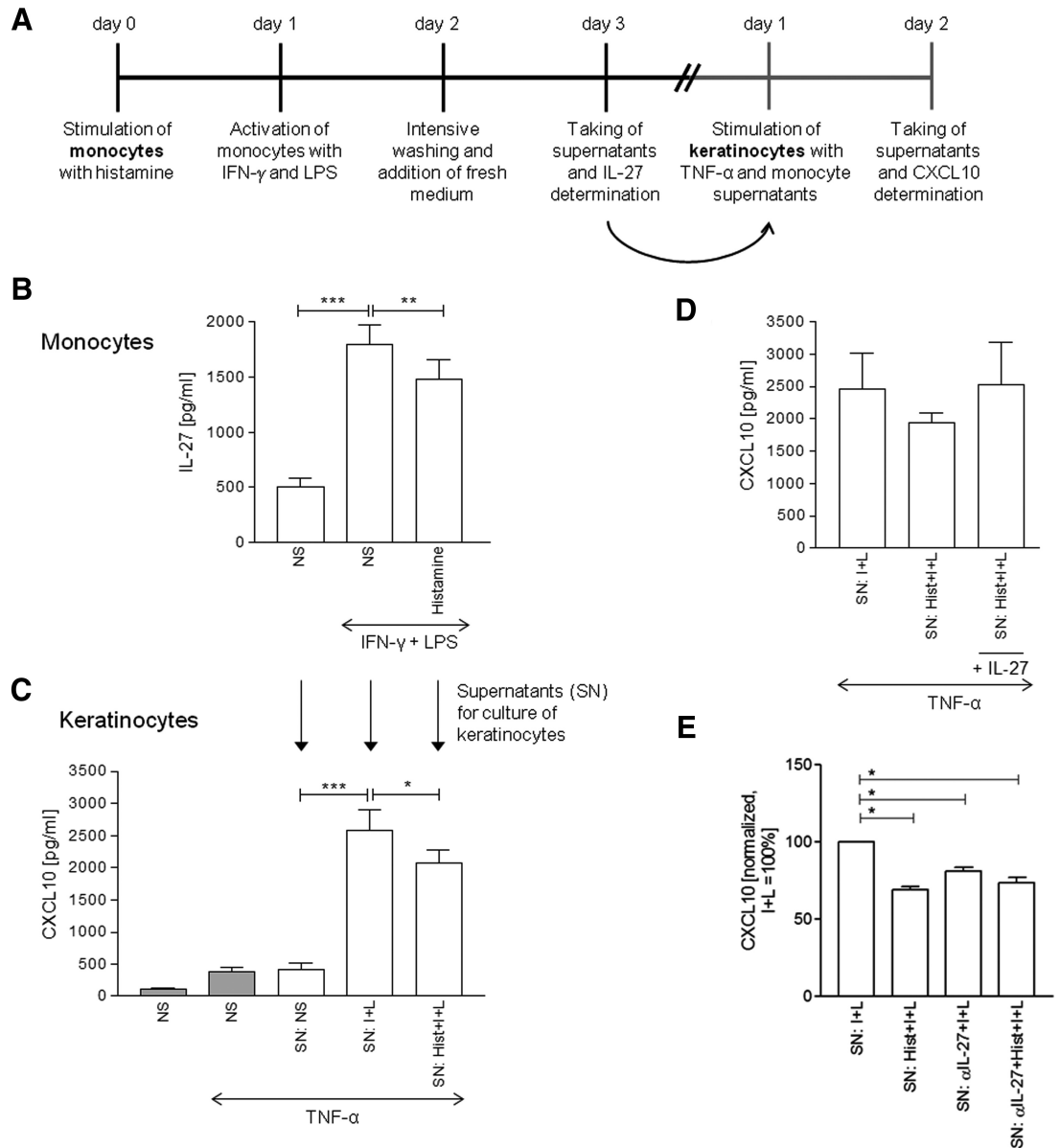
In line with our observation in human monocytes, higher IL-27 production was detected in BMDCs from $H_4R^{-/-}$ mice as compared with WT mice. BMDCs activated with TLR ligands responded more vigorously with IL-27 production when derived from $H_4R^{-/-}$ mice as compared with WT mice. For other inflammatory cytokines, such as IL-6, IL-10, and TNF- α , no differences in their secretion levels in the $H_4R^{-/-}$ mice were observed. Another study performed in the $H_4R^{-/-}$ showed, similarly to our results, no effect on TNF- α secretion but described decreased IL-6 production in $H_4R^{-/-}$ DCs after LPS activation [31]. However, in the mentioned study, the experiments were

performed with spleen-derived DCs, cells that might react in a different manner than the BMDCs used in this study.

To elucidate the involved histamine receptor subtypes, we used selective ligands at the four known receptors. We observed a down-regulation of IL-27 after treatment with H_2R and H_4R agonists; however, a block of the histamine effect was only seen upon pretreatment with the H_2R antagonist but not the H_4R antagonist. There is evidence that the used H_4R agonist (4-methylhistamine) also has weak binding potential at the H_2R [32]; however, this H_2R binding does not seem to play a big role in our study, as 4-methylhistamine was blocked by a H_4R antagonist, indicating a H_4R -mediated action. The observation that the H_4R antagonist did not block the action of histamine might be explained by the assumption that histamine mainly down-regulates IL-27 via the H_2R and that the H_4R only plays an important role in the absence of H_2R signaling. The involvement of the H_4R is supported further by the observation that in BMDCs of $H_4R^{-/-}$ mice, higher levels of IL-27 were detected—in the presence or absence of histamine. Nevertheless, histamine reduced IL-27 expression in $H_4R^{-/-}$ mice and WT mice, indicating that also, in the mouse system, two histamine receptors are activated: the H_4R and presumably, the H_2R . Moreover, the involvement of the H_2R and H_4R in the down-regulation of IL-27 is in concordance with our previous findings about the histamine-mediated down-regulation of IL-12, a member of the same cytokine family [4].

The suppression of IL-27 by histamine stimulation was observed consistently; however, the reduction of IL-27 protein levels was only weak. Therefore, we attempted to prove that IL-27

Figure 7. Human primary KCs incubated with histamine-stimulated monocyte supernatant secrete less CXCL10. As depicted in the experimental setup, (A) monocytes were stimulated for 24 h with histamine, followed by activation with IFN- γ and LPS for 24 h. After intensive washing, the cells were incubated with fresh medium, and 24 h later, the supernatants were taken. The secretion of IL-27 into the cell supernatant was decreased in histamine-prestimulated cells (B). The same supernatants were used for the culture of KCs in combination with TNF- α stimulation. The supernatants that showed a high level of IL-27 (B) induced a high production of CXCL10 (C), whereas in histamine-prestimulated samples showing lower IL-27 levels (B), the production of CXCL10 was impaired (C). I, IFN- γ ; L, LPS; Hist, histamine. External addition of hrIL-27 to the KC culture restored CXCL10 production (D). Incubation of monocytes with an IL-27 neutralizing antibody resulted in similar reduction of CXCL10 production as treatment of monocytes with histamine. The combination of both treatments did not further reduce CXCL10 secretion (E). Mean and SEM of seven, eight, four, and six independent experiments are shown in B, C, D, and E, respectively.



down-regulation is of functional consequence. We investigated the effect of IL-27 on KCs, as KCs represent the predominant cell type in the skin and play a major role in the pathogenesis of inflammatory skin diseases. Recently, we showed that IL-27 is expressed in inflamed skin and that it induces chemokine production in KCs, in particular, CXCL10 [17]. Here, we showed that KCs stimulated with LPS-treated monocyte supernatants, which contain high levels of IL-27, produce high levels of the chemokine CXCL10. In contrast, KCs incubated with the supernatant of histamine-prestimulated monocytes produced lower levels of CXCL10. The addition of hrIL-27 to the histamine-prestimulated monocyte supernatants, showing low IL-27 content, could restore high chemokine production in KCs. The inhibition of IL-27, using a neutralizing antibody, resulted in a similar effect, as the down-regulation of IL-27 by histamine. These results indicate that histamine-induced down-regulation of IL-27 results in decreased activation of KCs. In context with a study showing the correlation

of IL-27 serum levels with the severity of psoriasis and the production of IFN- γ [18], our findings might imply that histamine diminishes inflammation in such diseases via the H₂R and H₄R and that H₂R and H₄R agonists might be beneficial in diseases with predominant Th1 cytokine milieu, such as psoriasis and chronic atopic dermatitis. Moreover, as the IL-27R is widely expressed on immune cells different from KCs, the responses on cytokine secretion in these cells might, in general, dampen immune responses, contributing to improvement of disease severity. Of great interest in this regard is the role of IL-27 in promoting Th1 responses, which was shown by human in vitro studies [12] and in mouse models [15, 16]. In regard to these observations, IL-27 seems to be, together with IL-12, of importance in the switch from the mainly Th2-mediated to a Th1-dominated milieu, which is observed during the development from acute to chronic atopic dermatitis. Our study implies that histamine interferes with this process by down-regulating IL-27 (as shown in this study) and

IL-12 [4]. Histamine could thereby interfere with the switch from a Th2- to a Th1-dominated milieu, preventing the chronification of atopic dermatitis and decreasing the severity of psoriasis.

Concluding, our study provides new evidence for a role of histamine in the regulation of IL-27 production, as shown by in vitro assays using human monocytes. Therefore, histamine receptor ligands, especially at the H₂R or combined H₂R and H₄R agonists, might be beneficial in the treatment of inflammatory skin diseases, such as chronic atopic dermatitis and psoriasis.

AUTHORSHIP

M.G., M.K., R.G., T.W., and W.B. conceived of and designed the experiments. B.K., H.B., and M.G. performed the experiments. R.L.T. provided H4R^{-/-} mice and was involved in study design. All authors were involved in the analysis and interpretation of the data. M.G. and R.G. wrote the paper.

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REFERENCES

- O'Mahony, L., Akdis, M., Akdis, C. A. (2011) Regulation of the immune response and inflammation by histamine and histaminereceptors. *J. Allergy Clin. Immunol.* **128**, 1153–1162.
- Tiligada, E., Zampeli, E., Sander, K., Stark, H. (2009) Histamine H3 and H4 receptors as novel drug targets. *Expert Opin. Invest. Drugs* **18**, 1519–1531.
- Hofstra, C. L., Desai, P. J., Thurmond, R. L., Fung-Leung, W. P. (2003) Histamine H4 receptor mediates chemotaxis and calcium mobilization of mast cells. *J. Pharmacol. Exp. Ther.* **305**, 1212–1221.
- Gutzmer, R., Diestel, C., Mommert, S., Kother, B., Stark, H., Wittmann, M., Werfel, T. (2005) Histamine H4 receptor stimulation suppresses IL-12p70 production and mediates chemotaxis in human monocyte-derived dendritic cells. *J. Immunol.* **174**, 5224–5232.
- Ling, P., Ngo, K., Nguyen, S., Thurmond, R. L., Edwards, J. P., Karlsson, L., Fung-Leung, W. P. (2004) Histamine H4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *Br. J. Pharmacol.* **142**, 161–171.
- Gantner, F., Sakai, K., Tusche, M. W., Cruikshank, W. W., Center, D. M., Bacon, K. B. (2002) Histamine h(4) and h(2) receptors control histamine-induced interleukin-16 release from human CD8(+) T cells. *J. Pharmacol. Exp. Ther.* **303**, 300–307.
- Gutzmer, R., Mommert, S., Gschwandtner, M., Zwingmann, K., Stark, H., Werfel, T. (2009) The histamine H4 receptor is functionally expressed on T(H)2 cells. *J. Allergy Clin. Immunol.* **123**, 619–625.
- Dijkstra, D., Stark, H., Chazot, P.L., Shenton, F.C., Leurs, R., Werfel, T., Gutzmer, R. (2008) Human inflammatory dendritic epidermal cells express a functional histamine H4 receptor. *J. Invest. Dermatol.* **128**, 1696–1703.
- Gschwandtner, M., Rossbach, K., Dijkstra, D., Baumer, W., Kietzmann, M., Stark, H., Werfel, T., Gutzmer, R. (2010) Murine and human Langerhans cells express a functional histamine H receptor: modulation of cell migration and function. *Allergy* **65**, 840–849.
- Devergne, O., Hummel, M., Koeppen, H., Le Beau, M. M., Nathanson, E. C., Kieff, E., Birkenbach, M. (1996) A novel interleukin-12 p40-related protein induced by latent Epstein-Barr virus infection in B lymphocytes. *J. Virol.* **70**, 1143–1153.
- Devergne, O., Birkenbach, M., Kieff, E. (1997) Epstein-Barr virus-induced gene 3 and the p35 subunit of interleukin 12 form a novel heterodimeric hematopoietin. *Proc. Natl. Acad. Sci. USA* **94**, 12041–12046.
- Pflanz, S., Timans, J. C., Cheung, J., Rosales, R., Kanzler, H., Gilbert, J., Hibbert, L., Churakova, T., Travis, M., Vaisberg, E., Blumenschein, W. M., Mattson, J. D., Wagner, J. L., To, W., Zurawski, S., McClanahan, T. K., Gorman, D. M., Bazan, J. F., de Waal Malefyt, R., Rennick, D., Kastelein, R. A. (2002) IL-27, a heterodimeric cytokine composed of EB13 and p28 protein, induces proliferation of naive CD4(+) T cells. *Immunity* **16**, 779–790.
- Wirtz, S., Becker, C., Fantini, M. C., Nieuwenhuis, E. E., Tubbe, I., Galle, P. R., Schild, H. J., Birkenbach, M., Blumberg, R. S., Neurath, M. F. (2005) EBV-induced gene 3 transcription is induced by TLR signaling in primary dendritic cells via NF-κB activation. *J. Immunol.* **174**, 2814–2824.
- Pflanz, S., Hibbert, L., Mattson, J., Rosales, R., Vaisberg, E., Bazan, J. F., Phillips, J. H., McClanahan, T. K., de Waal, M. R., Kastelein, R. A. (2004) WSX-1 and glycoprotein 130 constitute a signal-transducing receptor for IL-27. *J. Immunol.* **172**, 2225–2231.
- Chen, Q., Ghilardi, N., Wang, H., Baker, T., Xie, M. H., Gurney, A., Grewal, I. S., de Sauvage, F. J. (2000) Development of Th1-type immune responses requires the type I cytokine receptor TCCR. *Nature* **407**, 916–920.
- Yoshida, H., Hamano, S., Senaldi, G., Covey, T., Faggioni, R., Mu, S., Xia, M., Wakeham, A. C., Nishina, H., Potter, J., Saris, C. J., Mak, T. W. (2001) WSX-1 is required for the initiation of Th1 responses and resistance to *L. major* infection. *Immunity* **15**, 569–578.
- Wittmann, M., Zeitvogel, J., Wang, D., Werfel, T. (2009) IL-27 is expressed in chronic human eczematous skin lesions and stimulates human keratinocytes. *J. Allergy Clin. Immunol.* **124**, 81–89.
- Shibata, S., Tada, Y., Kanda, N., Nashiro, K., Kamata, M., Karakawa, M., Miyagaki, T., Kai, H., Saeki, H., Shirakata, Y., Watanabe, S., Tamaki, K., Sato, S. (2010) Possible roles of IL-27 in the pathogenesis of psoriasis. *J. Invest. Dermatol.* **130**, 1034–1039.
- Krogstad, A. L., Lonnroth, P., Larson, G., Wallin, B. G. (1997) Increased interstitial histamine concentration in the psoriatic plaque. *J. Invest. Dermatol.* **109**, 632–635.
- Ruzicka, T., Simmet, T., Peskar, B. A., Ring, J. (1986) Skin levels of arachidonic acid-derived inflammatory mediators and histamine in atopic dermatitis and psoriasis. *J. Invest. Dermatol.* **86**, 105–108.
- Morath, S., Geyer, A., Hartung, T. (2001) Structure-function relationship of cytokine induction by lipoteichoic acid from *Staphylococcus aureus*. *J. Exp. Med.* **193**, 393–397.
- Baumer, W., Wendorff, S., Gutzmer, R., Werfel, T., Dijkstra, D., Chazot, P., Stark, H., Kietzmann, M. (2008) Histamine H4 receptors modulate dendritic cell migration through skin—immunomodulatory role of histamine. *Allergy* **63**, 1387–1394.
- Wittmann, M., Zwierner, J., Larsson, V. A., Kirchhoff, K., Begemann, G., Kapp, A., Götz, O., Werfel, T. (1999) C5a suppresses the production of IL-12 by IFN-γ-primed and lipopolysaccharide-challenged human monocytes. *J. Immunol.* **162**, 6763–6769.
- Horst, B., Borck, H., Thurmond, R., Grosch, S., Diel, F. (2006) STAT1 phosphorylation and cleavage is regulated by the histamine (H4) receptor in human atopic and non-atopic lymphocytes. *Int. Immunopharmacol.* **6**, 1577–1585.
- Morse, K. L., Behan, J., Laz, T. M., West Jr., R. E., Greenfeder, S. A., Anthes, J. C., Umland, S., Wan, Y., Hipkin, R. W., Gonsiorek, W., Shin, N., Gustafson, E. L., Qiao, X., Wang, S., Hedrick, J. A., Greene, J., Bayne, M., Monsma Jr., F. J. (2001) HCloning and characterization of a novel human histamine receptor. *J. Pharmacol. Exp. Ther.* **296**, 1058–1066.
- Molle, C., Goldman, M., Goriely, S. (2010) Critical role of the IFN-stimulated gene factor 3 complex in TLR-mediated IL-27p28 gene expression revealing a two-step activation process. *J. Immunol.* **184**, 1784–1792.
- May, M. J., Ghosh, S. (1998) Signal transduction through NF-κB. *Immunol. Today* **19**, 80–88.
- Foey, A. D., Parry, S. L., Williams, L. M., Feldmann, M., Foxwell, B. M., Brennan, F. M. (1998) Regulation of monocyte IL-10 synthesis by endogenous IL-1 and TNF-α: role of the p38 and p42/44 mitogen-activated protein kinases. *J. Immunol.* **160**, 920–928.
- Kimura, A., Naka, T., Muta, T., Takeuchi, O., Akira, S., Kawase, I., Kishimoto, T. (2005) Suppressor of cytokine signaling-1 selectively inhibits LPS-induced IL-6 production by regulating JAK-STAT. *Proc. Natl. Acad. Sci. USA* **102**, 17089–17094.
- Van der Bruggen, T., Nijenhuis, S., van, R. E., Verhoef, J., van Asbeck, B. S. (1999) Lipopolysaccharide-induced tumor necrosis factor α production by human monocytes involves the raf-1/MEK1-MEK2/ERK1-ERK2 pathway. *Infect. Immun.* **67**, 3824–3829.
- Dunford, P. J., O'Donnell, N., Riley, J. P., Williams, K. N., Karlsson, L., Thurmond, R. L. (2006) The histamine H4 receptor mediates allergic airway inflammation by regulating the activation of CD4+ T cells. *J. Immunol.* **176**, 7062–7070.
- Lim, H. D., Van Rijn, R. M., Ling, P., Bakker, R. A., Thurmond, R. L., Leurs, R. (2005) Evaluation of histamine H1-, H2-, and H3-receptor ligands at the human histamine H4 receptor: identification of 4-methyl-histamine as the first potent and selective H4 receptor agonist. *J. Pharmacol. Exp. Ther.* **314**, 1310–1321.

KEY WORDS:

CXCL10 · H₂ receptor · H₄ receptor · keratinocyte · monocyte