

Histamine receptor 2 modifies dendritic cell responses to microbial ligands

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Background: The induction of tolerance and protective immunity to microbes is significantly influenced by host- and microbiota-derived metabolites, such as histamine.

Objective: We sought to identify the molecular mechanisms for histamine-mediated modulation of pattern recognition receptor signaling.

Methods: Human monocyte-derived dendritic cells (MDDCs), myeloid dendritic cells, and plasmacytoid dendritic cells were examined. Cytokine secretion, gene expression, and transcription factor activation were measured after stimulation with microbial ligands and histamine. Histamine receptor 2 (H₂R)-deficient mice, histamine receptors, and their signaling pathways were investigated.

Results: Histamine suppressed MDDC chemokine and proinflammatory cytokine secretion, nuclear factor κ B and activator protein 1 activation, mitogen-activated protein kinase phosphorylation, and T_H1 polarization of naive lymphocytes, whereas IL-10 secretion was enhanced in response to LPS and Pam3Cys. Histamine also suppressed LPS-induced myeloid dendritic cell TNF- α secretion and suppressed CpG-induced plasmacytoid dendritic cell IFN- α gene expression. H₂R signaling through cyclic AMP and exchange protein directly activated by cyclic AMP was required for the histamine effect on LPS-induced MDDC responses. *Lactobacillus rhamnosus*, which secretes histamine, significantly suppressed Peyer patch IL-2, IL-4, IL-5, IL-12, TNF- α , and GM-CSF secretion in wild-type but not H₂R-deficient animals.

Conclusion: Both host- and microbiota-derived histamine significantly alter the innate immune response to microbes through H₂R. (J Allergy Clin Immunol 2013;■■■■:■■■■-■■■■.)

Key words: Dendritic cell, microbiota, pattern recognition receptors, histamine, histamine receptors, nuclear factor κ B, cyclic AMP, *Lactobacillus rhamnosus*

Activation of the innate immune system to microbial ligands initiates protective and tolerogenic signaling networks, culminating in adaptive responses that can play a role in allergic sensitization.^{1,2} The innate immune response depends on the recognition of microbe-associated molecular patterns by pattern recognition receptors (PRRs) on host cells, such as dendritic cells (DCs). Differential binding of microbe-associated molecular patterns to PRRs leads to a complex array of interconnecting intracellular signaling pathways, which influence cellular metabolism, cytokine and chemokine secretion, antigen presentation, and cell-surface costimulatory and inhibitory molecule expression. In addition, microenvironmental factors, such as microbe- or host-derived metabolites, have profound effects on the polarization of PRR responses. This can clearly be observed within the gastrointestinal tract, whereby metabolites, such as retinoic acid or short-chain fatty acids, shape the intestinal innate and adaptive immune reactivity to the vast array of microbial and dietary antigens.^{3,4}

Toll-like receptors (TLRs) are one family of PRRs that are essential for protection against infection; however, inappropriate regulation of TLR responses contributes to acute and chronic inflammation, as well as to autoimmune and allergic diseases.^{5,6} Ligation of TLRs activates transcription factors, thus driving the secretion of proinflammatory and regulatory mediators. For example, DC TLR2 activation by the commensal microbe *Bifidobacterium infantis* 35624 leads to IL-10 secretion and induction of retinoic acid metabolism, resulting in polarization of naive lymphocytes into forkhead box protein 3 (Foxp3)-positive regulatory T cells.⁷ The TLR-signaling cascade is regulated at many different levels, including splice variants of adaptor proteins, ubiquitin ligases, deubiquitinases, kinase regulators, transcriptional regulators, or micro-RNA.⁸ In addition, external metabolites can influence TLR sensitivity by interacting at several points, such as the inhibition of LPS-induced I κ B degradation by short-chain fatty acids.⁹

Histamine (2-[1H-imidazo-4-yl]ethanamine) is a biogenic amine that influences multiple cell types, including DCs, natural killer cells, epithelial cells, T lymphocytes, and B lymphocytes.¹⁰ Histamine is immediately released after activation of mast cells and basophils, whereas lymphocytes, monocytes, and DCs can synthesize and secrete histamine after stimulation.¹¹ Several bacterial strains can also synthesize histamine.¹² Histamine and its 4 receptors represent a complex system of immunoregulation with distinct effects depending on receptor subtypes and their differential expression.¹³

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Supported by Swiss National Foundation grants (project nos. 310030-127356 and 32003027618/1), the Christine Kühne-Center for Allergy Research and Education (CK-CARE), European Union research grants, COST Action BM0806, and European Union Marie Curie grants.

Disclosure of potential conflict of interest: T. Watanabe has received grants and travel support from MEXT. R. Lauener has received grants from the Kühne Foundation and the European Union and serves on advisory boards of Merck Sharp & Dohme, Novartis, Nestlé, and Menarini. C. A. Akdis has consultant arrangements with Actellion, Aventis, Stallergenes, Allergopharma, and Circacia; is employed by the Swiss Institute of Allergy and Asthma Research; and has received grants from Novartis, PREDICTA: European Commission's Seventh Framework, the Swiss National Science Foundation, MeDALL: European Commission's Seventh Framework Programme, and the Christine Kühne-Center for Allergy Research and Education. L. O'Mahony has received grants from the Swiss National Science Foundation and the European Union and has consultant arrangements with Alimentary Health Ltd. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication September 18, 2012; revised December 13, 2012; accepted for publication January 7, 2013.

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0091-6749/\$36.00

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<http://dx.doi.org/10.1016/j.jaci.2013.01.013>

Abbreviations used

AP-1: Activator protein 1
 cAMP: Cyclic AMP
 DC: Dendritic cell
 Epac: Exchange protein directly activated by cAMP
 Foxp3: Forkhead box protein 3
 H₁R: Histamine 1 receptor
 H₂R: Histamine 2 receptor
 H₄R: Histamine 4 receptor
 mDC: Myeloid dendritic cell
 MDDC: Monocyte-derived dendritic cell
 NF-κB: Nuclear factor κB
 pDC: Plasmacytoid dendritic cell
 PKA: Protein kinase A
 PMA: Phorbol 12-myristate 13-acetate
 PRR: Pattern recognition receptor
 TLR: Toll-like receptor

Maintenance of mucosal homeostasis is heavily dependent on appropriate sampling and processing of microbial ligands by the innate immune system, particularly DCs. Here we investigated the molecular mechanisms by which histamine derived from the host or the microbiota modulates DC and mucosal immunologic responses and report that histamine significantly alters TLR signaling in DCs, which is mediated by the histamine 2 receptor (H₂R)–cyclic AMP (cAMP)–exchange protein directly activated by cAMP (Epac) signaling cascade. Furthermore, suppression of Peyer patch cytokine secretion by the commensal microbe *Lactobacillus rhamnosus* is abrogated in H₂R-deficient animals.

METHODS

For additional information, see the Methods section in this article's Online Repository at www.jacionline.org.

Reagents and cell isolation

LPS and histamine were obtained from Sigma (Buchs, Switzerland) and used at concentrations of 500 ng/mL and 1×10^{-5} mol/L, respectively. In addition, the histamine 1 receptor (H₁R) antagonist diphenhydramine, the H₂R antagonist famotidine, the adenylate cyclase activator forskolin (10^{-5} mol/L), the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (10^{-7} mol/L), and cycloheximide (10^{-4} mol/L) were obtained from Sigma, whereas the histamine 4 receptor (H₄R) antagonist JNJ-777120 was a gift from Dr Robin Thurmond (Johnson & Johnson Pharmaceutical Research & Development, San Diego, Calif). Inhibitors were added 30 minutes before stimulation. Pam3Cys and cAMP were obtained from Calbiochem (Merck KGaA, Darmstadt, Germany). CpG ($1 \mu\text{mol/L}$) was obtained from Microsynth (Balgach, Switzerland). Human monocyte-derived dendritic cells (MDDCs) were differentiated with 1000 IU/mL GM-CSF (Peprotech, London, United Kingdom) and 1000 IU/mL IL-4 (Novartis, Basel, Switzerland) from purified CD14⁺ cells by using magnetic cell sorting separation (Miltenyi Biotec, Bergisch Gladbach, Germany). CD1c⁺ myeloid dendritic cells (mDCs) were isolated by using magnetic cell sorting separation (Miltenyi Biotec). Plasmacytoid dendritic cells (pDCs) were isolated with anti-CD304 antibody–coupled beads (Miltenyi Biotec), followed by fluorescence-activated cell sorting for CD123⁺CD303⁺ cells (Aria Cell Sorter; BD Biosciences, Franklin Lakes, NJ). pDCs were cultured in the presence of 10 ng/mL IL-3. THP-1–XBlue cells containing the NF-κB/activator protein 1 (AP-1) reporter system were obtained from InvivoGen (Carlsbad, Calif). All cells were cultured in RPMI containing 10% FCS at 37°C and 5% CO₂.

Quantification of cytokines, chemokines, cAMP, histamine, and NF-κB phosphorylation

Mitogen-activated protein kinase phosphorylation, cytokines, and chemokines were quantified with the BioPlex Multiplex Suspension Array System (Bio-Rad Laboratories, Reinach, Switzerland). cAMP was quantified by using the cAMP Direct Biotrak EIA (Amersham Biosciences, Freiburg, Germany). Histamine was measured by means of ELISA (Cusabio, Hoelzel Diagnostika, Cologne, Germany), and NF-κB phosphorylation was measured with phospho-specific antibodies (BD Biosciences) and flow cytometry.

Flow cytometric analysis

All flow cytometric analyses were performed on the Gallios Flow Cytometer (Beckman Coulter, Brea, Calif). Anti-CD4, anti-CD25, anti-T-box transcription factor (T-bet), anti-GATA3, and anti-Foxp3 antibodies (eBioscience, Vienna, Austria) were used for T-cell staining. Labeled anti-human H₁R antibody was obtained from R&D Systems (Minneapolis, Minn). Anti-human H₂R (GeneTex; Lucerna-Chem, Luzern, Switzerland) and anti-human H₄R (Alomone Labs, Jerusalem, Israel) antibodies were labeled with DyLight Fluorescent Dyes (Thermo Scientific, Schwerte, Germany).

RESULTS**Histamine modulates TLR-induced cytokine secretion from human DCs**

Human MDDCs were stimulated with LPS (TLR-4 ligand) or Pam3Cys (TLR-2 ligand) in the presence or absence of histamine. LPS-stimulated secretion of TNF-α, IL-12p70, and CXCL10 was significantly reduced when MDDCs were also exposed to histamine, whereas IL-10 secretion was enhanced (Fig 1, A). Histamine was used at a concentration of 1×10^{-5} mol/L, as indicated by dose-response studies (see Fig E1 in this article's Online Repository at www.jacionline.org). Gene expression profiling confirmed modulation of these cytokine and chemokine responses (see Fig E2 in this article's Online Repository at www.jacionline.org). The histamine-associated increase in IL-10 gene expression was substantially greater than the increase subsequently observed at the protein level, perhaps because of differential effects of histamine at early and late time points (ie, gene expression at 4 hours vs accumulated protein levels at 24 hours). Histamine also decreased Pam3Cys-stimulated TNF-α secretion and enhanced IL-10 secretion (Fig 1, B). Cycloheximide treatment of the MDDCs did not inhibit histamine-associated suppression of TNF-α but did block enhanced gene expression of IL-10, suggesting that new protein synthesis is only required for enhancement of IL-10 expression (see Fig E3 in this article's Online Repository at www.jacionline.org).

mDCs were directly isolated from human peripheral blood, and histamine suppressed LPS-stimulated mDC TNF-α secretion (Fig 1, C) and Pam3Cys-stimulated mDC TNF-α secretion (see Fig E4, A, in this article's Online Repository at www.jacionline.org). Histamine enhanced IL-10 secretion by TLR-stimulated mDCs (see Fig E4, B). Furthermore, we investigated the influence of histamine on pDC TLR reactivity. Because pDCs express a different repertoire of TLRs and cytokines compared with mDCs, we assessed IFN-α gene expression by CpG (TLR9 ligand)–activated pDCs (Fig 1, D). Histamine suppressed IFN-α gene expression but had no influence on IL-10 gene expression by CpG-stimulated pDCs (see Fig E4, C).

Naive autologous lymphocytes were coincubated with activated MDDCs to evaluate the functional significance of histamine on LPS-stimulated MDDCs. LPS-stimulated MDDCs induced T_H1 lymphocyte polarization, whereas LPS and histamine-stimulated

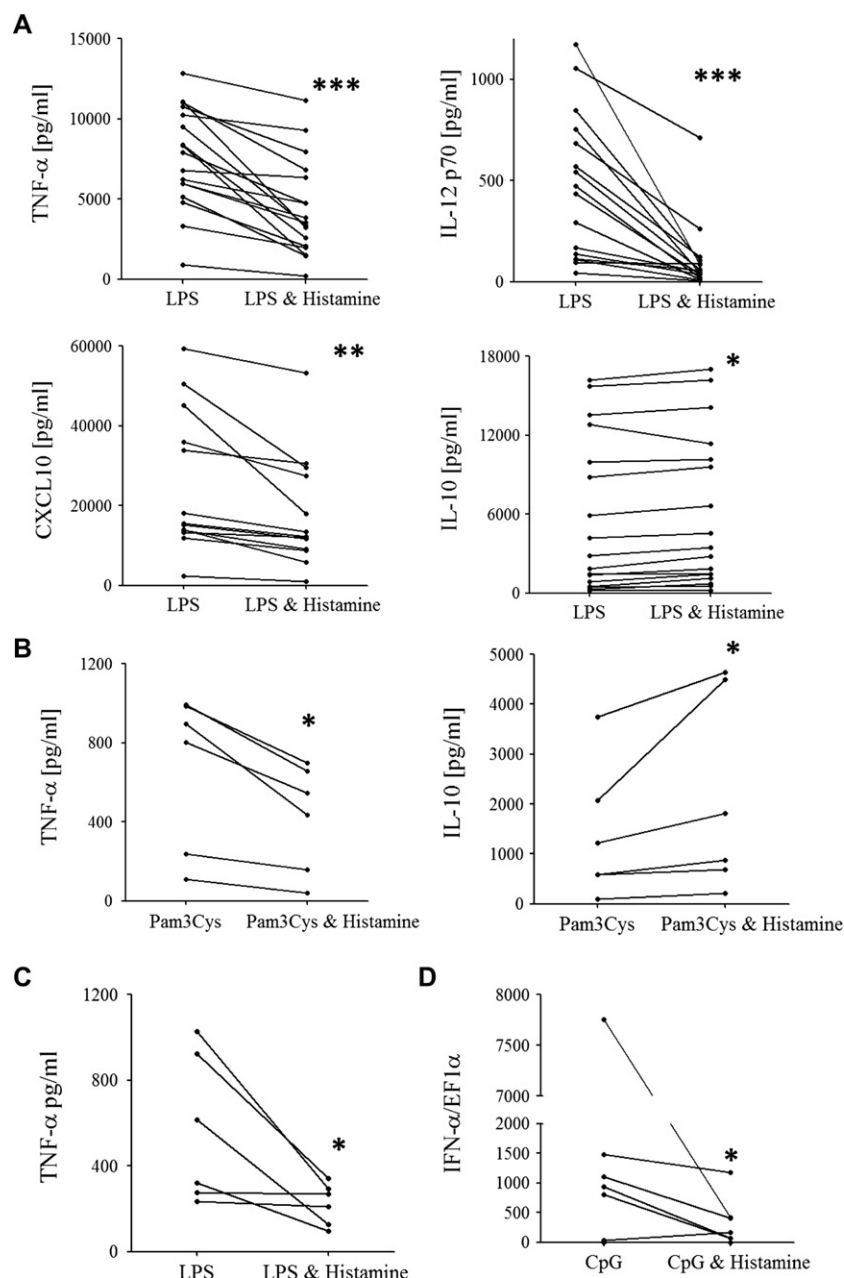


FIG 1. Histamine modulates cytokine secretion from TLR-stimulated DCs. **A**, Human MDDCs secreted less TNF- α , IL-12p70, and CXCL10 after LPS stimulation, whereas IL-10 secretion was increased, in the presence of histamine. **B**, MDDCs stimulated with Pam3Cys and histamine resulted in decreased TNF- α and enhanced IL-10 secretion. **C** and **D**, LPS-stimulated TNF- α secretion from mDCs was suppressed by histamine (Fig 1, C), whereas IFN- α gene expression in CpG-stimulated pDCs was also suppressed by histamine coinubation (Fig 1, D). Each line represents an individual donor. EF1 α , Elongation factor 1 α . * P < .05, ** P < .01, and *** P < .001.

MDDCs did not induce T_H1 polarization (Fig 2, A and B). T-cell proliferation was not altered and remained low (<10%) because of the lack of polyclonal stimuli. Lymphocyte GATA-3 (T_H2) expression and Foxp3 (regulatory T) expression were not significantly influenced by the addition of histamine (Fig 2, C).

Histamine modulates TLR-induced transcription factor activation

Triggering of TLRs activates transcription factors, such as nuclear factor κ B (NF- κ B) and AP-1. Therefore we used the

human monocyte cell line THP-1, which expresses histamine receptors to a similar extent as MDDCs. LPS-induced NF- κ B/AP-1 was significantly suppressed by histamine (Fig 3, A). Identical results were observed with Pam3Cys, intact gram-positive microbes, or intact gram-negative microbes (see Fig E5 in this article's Online Repository at www.jacionline.org). In addition, LPS-induced MDDC NF- κ B phosphorylation at serine 536 and serine 529 was reduced when histamine was present (Fig 3, B). However, histamine did not alter LPS-induced I κ B phosphorylation or degradation (Fig 3, C). Kinase phosphorylation after LPS stimulation of MDDCs was

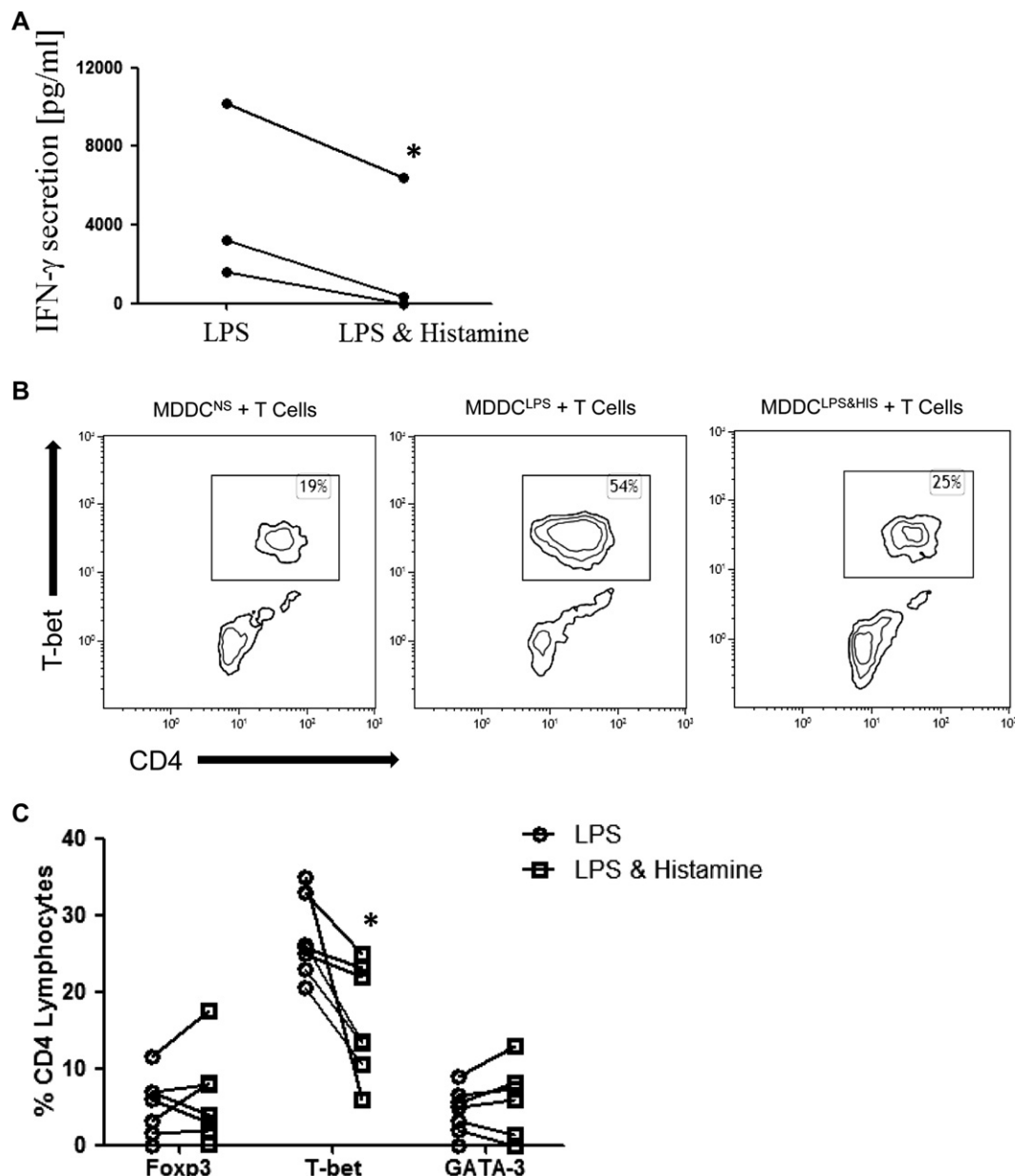


FIG 2. Histamine suppresses T_H1 lymphocyte polarization. **A** and **B**, Lymphocytes secreted IFN- γ and expressed T-box transcription factor (*T-bet*) after coculture with LPS-stimulated MDDCs. However, coculture of MDDCs with histamine significantly reduced IFN- γ secretion (Fig 2, **A**) and *T-bet* expression (Fig 2, **B**). **C**, GATA-3 and Foxp3 expression were not significantly altered. * $P < .05$.

significantly impaired by histamine, whereas phosphorylation of c-Jun (part of the AP-1 complex) was significantly reduced (Fig 3, **D**). Similar results were observed for Pam3Cys-stimulated MDDCs (see Fig E6 in this article's Online Repository at www.jacionline.org).

Inhibition of the serine/threonine protein kinase mammalian target of rapamycin with rapamycin did not alter suppression of TNF- α , IL-12, and CXCL10 secretion (Fig 4, **A**). In contrast, histamine no longer enhanced IL-10 secretion after mammalian target of rapamycin antagonism. In addition, we investigated whether epigenetic modifications might play a role. Using the histone deacetylation inhibitor trichostatin, histamine retained its suppressive activity on LPS-induced TNF- α , IL-12, and CXCL10 secretion, whereas IL-10 secretion was no longer significantly upregulated

by histamine (Fig 4, **A**). Finally, we assessed the potential role for phosphoinositide 3-kinases by blocking these kinases with wortmannin or Ly-294. Neither inhibitor altered the histamine effects on IL-10, TNF- α , IL-12, or CXCL10 secretion (Fig 4, **B**).

H₂R mediates inhibition of the TLR-dependent inflammatory response

MDDCs express the H₁R, H₂R, and H₄R genes, whereas histamine 3 receptor gene expression was not detected (Fig 5, **A**). Flow cytometry confirmed cell-surface expression of these receptors (see Fig E7, **A**, in this article's Online Repository at www.jacionline.org). Interestingly, H₁R and H₄R gene expression was significantly decreased by LPS stimulation, whereas H₂R

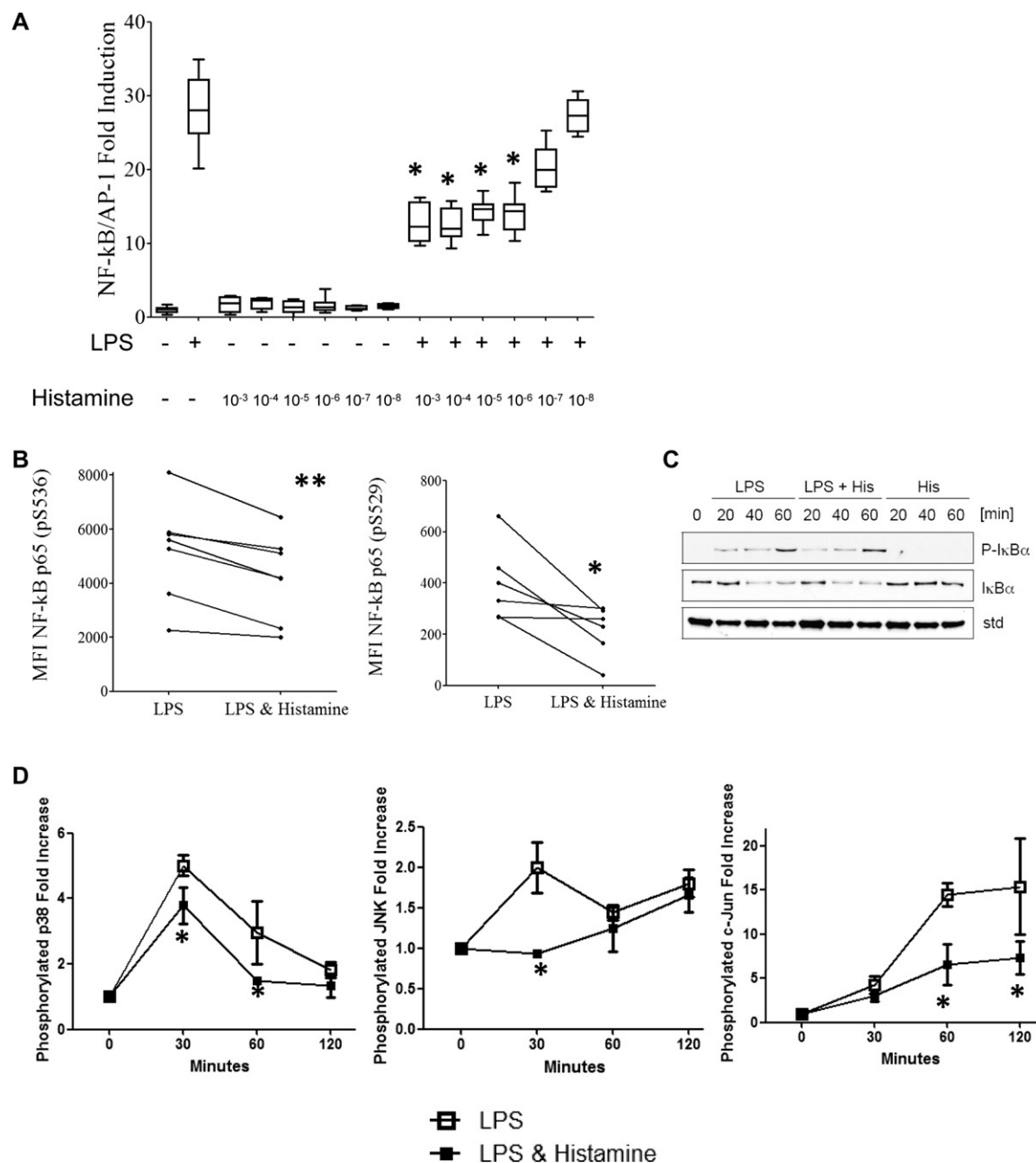


FIG 3. Histamine suppresses TLR-induced NF-κB and AP-1 activation. **A**, THP-1 cells stimulated with LPS in the presence of histamine displayed reduced NF-κB/AP-1 activity compared with that seen in cells stimulated with LPS alone. **B**, Histamine reduces TLR-induced NF-κB phosphorylation in MDDCs. *MFI*, Mean fluorescence intensity. **C**, Phosphorylation and degradation of IκB were unaltered by histamine coinubation. β-Actin staining is the loading control. **D**, Phosphorylation of p38, c-Jun N-terminal kinase (*JNK*), and c-Jun was significantly suppressed by histamine. **P* < .05 and ***P* < .01.

gene expression was significantly increased by LPS stimulation (see Fig E7, *B*). After neutralization with specific antagonists, only the H₂R antagonist famotidine reversed the histamine-mediated reduction of NF-κB/AP-1 activation (Fig 5, *B*). In addition, the H₂R agonist dimaprit replicated the effects of histamine. Furthermore, famotidine reversed the histamine-mediated suppression of MDDC TNF-α secretion in response to LPS (Fig 5, *C*). The suppression of IL-12 and CXCL-10 secretion was also reversed by famotidine, whereas IL-10 secretion returned to levels similar to those induced by LPS alone (results not shown).

cAMP and Epac mediate the regulatory effects of H₂R

H₂R activates adenylate cyclase to generate cAMP. MDDCs stimulated with LPS alone did not induce cAMP, whereas costimulation with histamine induced robust cAMP generation. This was reversed by famotidine (Fig 6, *A*). Stimulation of MDDCs with LPS in the presence of the adenylate cyclase activator forskolin or in the presence of externally added cAMP replicated the effect of histamine on suppressing TNF-α secretion (Fig 6, *B*).

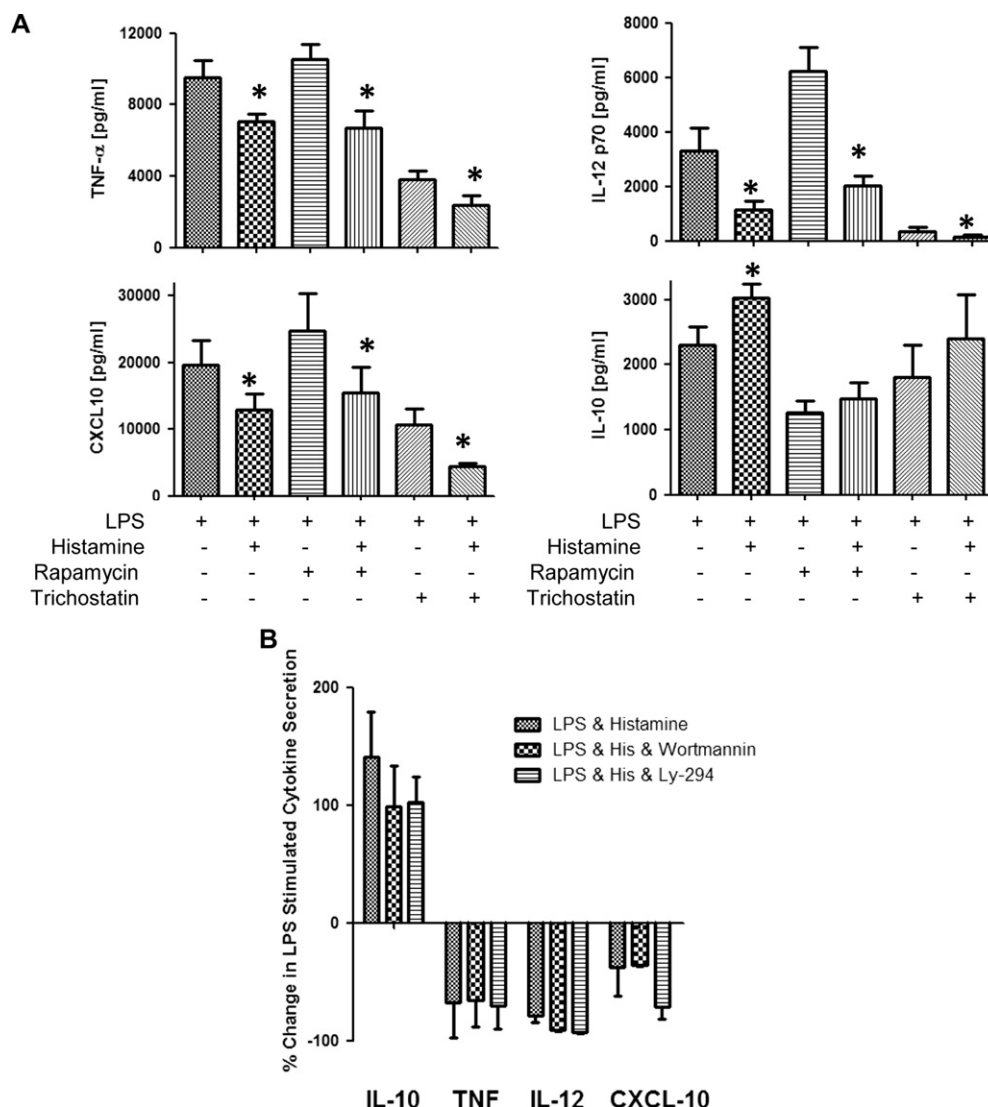


FIG 4. Histamine-associated signaling cascades. **A**, Inhibition of mammalian target of rapamycin signaling by rapamycin and histone deacetylation by trichostatin in MDDCs abrogated histamine-induced upregulation of IL-10, with no effect on histamine-induced suppression of TNF- α , IL-12, or CXCL10 ($n = 8$ donors). **B**, Neither of the phosphoinositide 3-kinase inhibitors altered the effects of histamine on IL-10, TNF- α , IL-12, or CXCL10 secretion. * $P < .05$.

In addition, the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine, which inhibits cAMP degradation, improved the suppressive effect of histamine (Fig 6, B). A similar effect was observed for the inhibition of IL-12 secretion by MDDCs (Fig 6, C) and for NF- κ B/AP-1 suppression in THP-1 cells (see Fig E8 in this article's Online Repository at www.jacionline.org). Therefore cAMP is a critical mediator of the histamine-H₂R effect on TLR activity.

cAMP activates protein kinase A (PKA) and Epac. The PKA antagonist H-89 did not reverse histamine-associated effects (Fig 6, D, and see Fig E9 in this article's Online Repository at www.jacionline.org). In contrast, the selective Epac agonist 8-CPT-cAMP replicated the influence of histamine on TNF- α , IL-12, and CXCL10, with a minor effect on IL-10 secretion (Fig 6, E), and inhibited NF- κ B/AP-1 activation (see Fig E9). Epac can further activate Rap1, and inhibition of Rap1 by the antagonist GGT1298 abolished the histamine-dependent upregulation of

IL-10 but had no effect on TNF- α , IL-12, or CXCL10 suppression (Fig 6, F).

Suppression of mucosal cytokine secretion by *L. rhamnosus* is H₂R dependent

L. rhamnosus is a commensal microbe commonly found within the gastrointestinal tract. We identified an *L. rhamnosus* strain that expresses histidine decarboxylase and secretes histamine *in vitro* (see Fig E10 in this article's Online Repository at www.jacionline.org). Oral administration of this bacterium to wild-type mice resulted in suppression of *Pseudomonas aeruginosa* lysate (containing a mixture of multiple TLR ligands), and phorbol 12-myristate 13-acetate (PMA)/ionomycin stimulated IL-10, IL-12, and TNF- α secretion by resected Peyer patch cells (Fig 7). Secretion of PMA/ionomycin-stimulated IL-2, IL-4, IL-5, and GM-CSF was also significantly suppressed

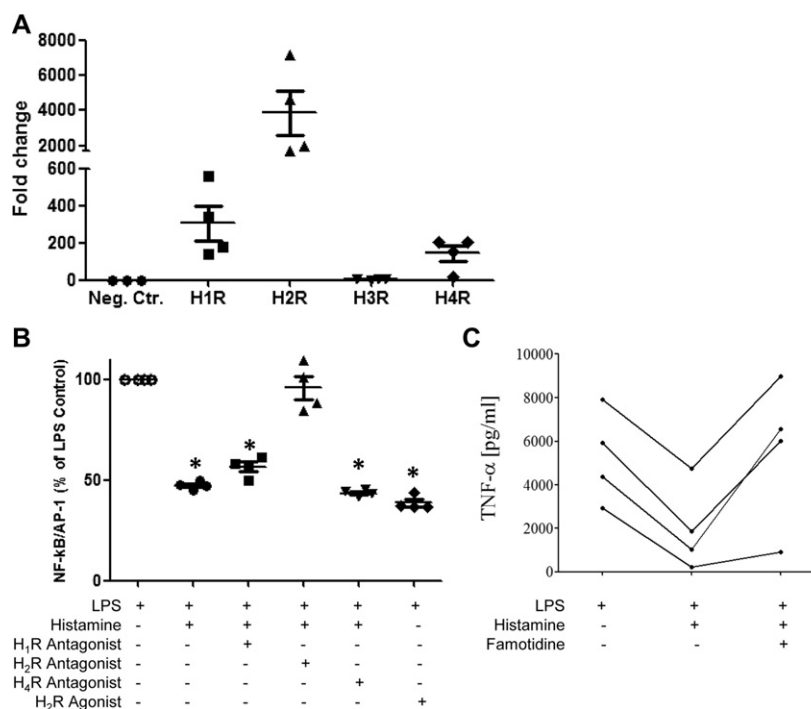


FIG 5. H₂R is responsible for the histamine modulatory effects. **A**, MDDCs express gene transcripts for H₁R, H₂R, and H₄R. **B**, Suppression of THP-1 cell NF-κB/AP-1 activation by histamine was blocked by an H₂R antagonist and replicated by an H₂R agonist. **C**, The H₂R antagonist famotidine also reversed the histamine-induced suppression of MDDC TNF-α secretion. **P* < .05.

(*P. aeruginosa* lysate did not induce these cytokines, see Fig E11 in this article's Online Repository at www.jacionline.org). However, simultaneous administration of this bacterium to H₂R knockout mice did not result in suppressed cytokine secretion (Fig 7). In addition, *L. rhamnosus* administration resulted in increased numbers of Foxp3⁺ lymphocytes within Peyer patches (day 0, 4.4% ± 0.52%; day 9, 6.1% ± 0.31%; *P* = .02), which was not observed in H₂R knockout animals (day 0, 5.4% ± 0.62%; day 9, 5.8% ± 0.26%; *P* = .65). Interestingly, resected Peyer patch cells from H₂R-deficient animals secrete less IL-2, IL-4, IL-10, IL-12, and TNF-α (but not less GM-CSF or IL-5) in response to PMA/ionomycin stimulation compared with that seen in Peyer patch cells from wild-type animals.

DISCUSSION

The signaling cascades induced by microbial ligand binding to TLRs are under tight control because inappropriate activation can lead to inflammatory or atopic diseases.^{14,15} Herein we describe H₂R as an important regulator of the DC TLR signaling pathways. The molecular mechanism involves cAMP activation of Epac, which leads to suppression of NF-κB activation and proinflammatory cytokine secretion. In addition, Rap1 is required for promoting IL-10 secretion, but Rap1 is not required for suppression of proinflammatory cytokine responses. The molecular interactions between the H₂R and TLR pathways are represented schematically in Fig 8.

DCs exposed to histamine have been previously shown to upregulate their antigen-presenting capacity and T_H1 polarization through H₁R, whereas activation of H₂R on DCs preferentially drives IL-10 secretion and, under certain circumstances, promotes T_H2 polarization.^{16,17} Our findings confirm and extend previous

observations concerning the effect of H₂R on DC cytokine signaling and blockade of T_H1 polarization. However, we did not observe promotion of T_H2 polarization. The role for H₂R in promoting T_H2 responses is controversial because additional studies have shown that H₂R inhibits T_H2 responses, which was clearly demonstrated for allergen-specific T cells in beekeepers, and H₂R is also upregulated on basophils during venom immunotherapy.^{18,19}

PKA was initially believed to be the main conduit for the effects of cAMP in immune cells. However, Epac is increasingly being recognized as another mediator of cAMP activity. PKA and Epac have been shown to induce excessive, independent, and even opposite effects inside the same cell.^{20,21} Our finding that Epac, but not PKA, is required for the histamine-mediated inhibition of DC NF-κB activation and proinflammatory cytokine secretion provides further support for specifically targeting Epac with selective compounds for the inhibition of exuberant TLR and inflammatory reactivity.

Inappropriate PRR signaling has been associated with multiple inflammatory and infectious diseases, particularly those of mucosal tissues.²²⁻²⁴ We demonstrate here that H₂R modulates the human DC response to microbial ligands by reducing their proinflammatory response. Under homeostatic conditions, the dampening of proinflammatory responses to nonpathogenic challenge could be considered protective, whereas excessive suppression of DC responses to infectious agents or cancer cells might be detrimental, resulting in immune evasion and pathogen persistence. Conflicting results have been reported regarding the role for histamine and histamine receptors in influencing protection against infectious agents. Blockade of H₂R impairs optimal innate immune responses during severe murine bacterial sepsis, and signaling through H₂R is required for control of *Yersinia enterocolitica* infection; in contrast,

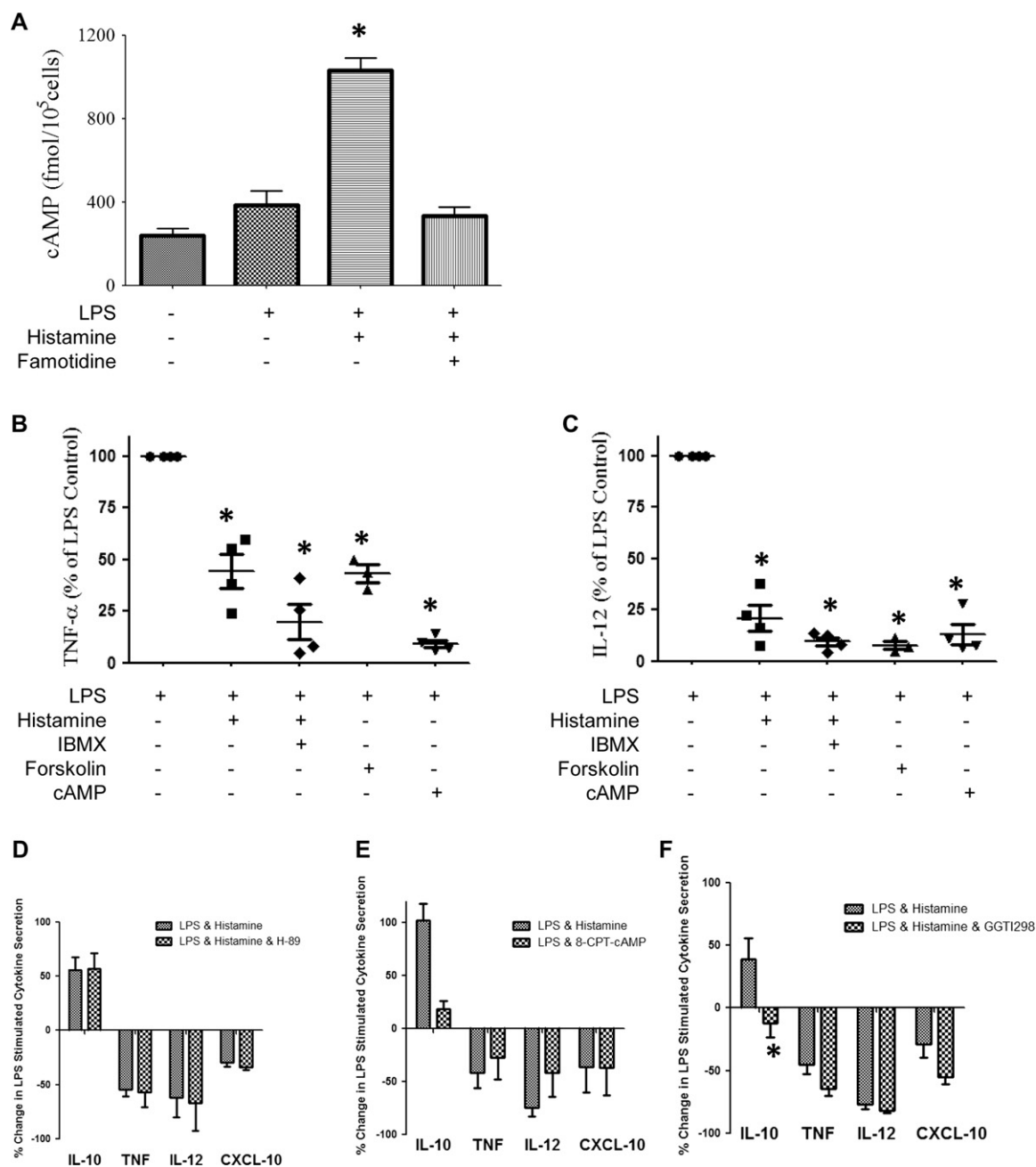


FIG 6. H₂R intracellular signaling requires cAMP and Epac. **A**, Histamine increased cAMP levels in MDDCs through H₂R. **B** and **C**, Histamine-associated suppression of LPS-induced MDDC TNF- α (Fig 6, **B**) and IL-12 (Fig 6, **C**) secretion was replicated by the cAMP inducer forskolin or exogenous cAMP and was improved by the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX). **D**, The PKA inhibitor H-89 had no effect on cytokine secretion. **E**, Epac activation reduced TNF- α , IL-12, and CXCL10 secretion from LPS-stimulated MDDCs but did not significantly enhance IL-10 secretion. **F**, Inhibition of Rap1 signaling by GGT1298 in MDDCs abrogated histamine-induced upregulation of IL-10. * $P < .05$ ($n = 4$ donors).

histamine-mediated signaling contributes to malaria pathogenesis.²⁵⁻²⁷ Taken together, it is clear that histamine and H₂R are intimately connected to the fine tuning of innate immune responses to microbes and appropriate PRR signaling. However, the microenvironmental or genetic polymorphisms that influence H₂R expression, activity, or both remain to be determined.

It is now appreciated that the metabolic activity of the microbiome modulates the immunoregulatory functions of the host. A number of bacterial strains are recognized to secrete

histamine, and one report has recently demonstrated that histamine secretion by a *Lactobacillus reuteri* strain modulates TLR2-induced TNF- α secretion *in vitro*.²⁸ However, the role played by microbe-derived histamine in homeostatic or pathologic mucosal immunoreactivity is not well characterized or understood. Our data suggest that H₂R is important in the immunoregulatory activity associated with a histamine-producing *L. rhamnosus* microbe. Baseline cytokine responses to microbial ligand stimulation were similar between H₂R knockout and wild-type animals; however,

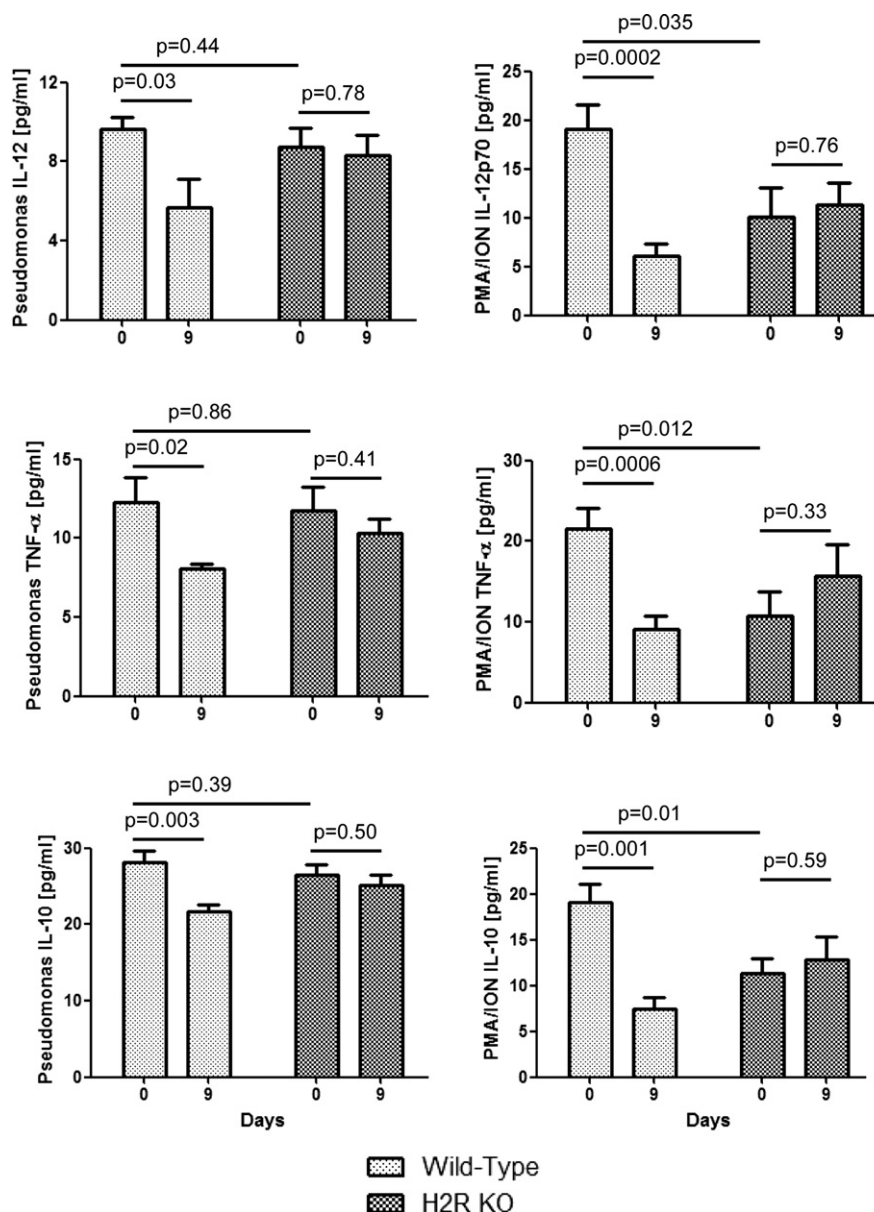


FIG 7. Differential effects of *L. rhamnosus* administration to wild-type and H₂R knockout (KO) mice. Oral administration of *L. rhamnosus* to wild-type animals (n = 10) significantly reduces Peyer patch cytokine secretion, which was not observed in H₂R knockout animals (n = 8). Results are expressed as means plus SEMs before (day 0) and after (day 9) feeding.

PMA/ionomycin-stimulated H₂R knockout Peyer patch IL-2, IL-4, IL-10, IL-12, and TNF-α secretion before *L. rhamnosus* administration was significantly lower than that observed for wild-type animals. The phorbol ester and calcium ionophore stimulation of cells is mediated by molecular mechanisms distinct from those stimulated by microbial ligands. In addition, PMA/ionomycin can activate cellular subsets that are not activated by *Pseudomonas* species lysates. Thus the differences in cytokine secretion at baseline between H₂R knockout and wild-type animals suggests that H₂R is an important regulatory receptor that influences mucosal immune responses through multiple mechanisms with potentially divergent effects on individual cell subpopulations. The PMA/ionomycin data are consistent with a previous report in which H₂R knockout animals were less susceptible to experimental allergic encephalomyelitis caused by

CCL2-mediated inhibition of T_H1 cytokine secretion.²⁹ Ultimately, the relative protective or pathologic importance of H₂R might largely depend on the manner by which the immune system is activated (ie, antigen-specific versus PRR stimulation), the species (ie, mouse vs human), and the stimulation conditions (eg, the use of adjuvants or pre-existing local inflammatory disease). A more in-depth dissection of the molecular and cellular alterations within the mucosa of H₂R knockout animals will likely lead to a better understanding of the precise role played by this receptor in mucosal immune regulation.

In conclusion, histamine modulates the TLR-induced activation of DCs through H₂R-cAMP-Epac. In addition, H₂R is required for the Peyer patch immunomodulatory activity of *L. rhamnosus*. Strategies that promote H₂R expression and activity

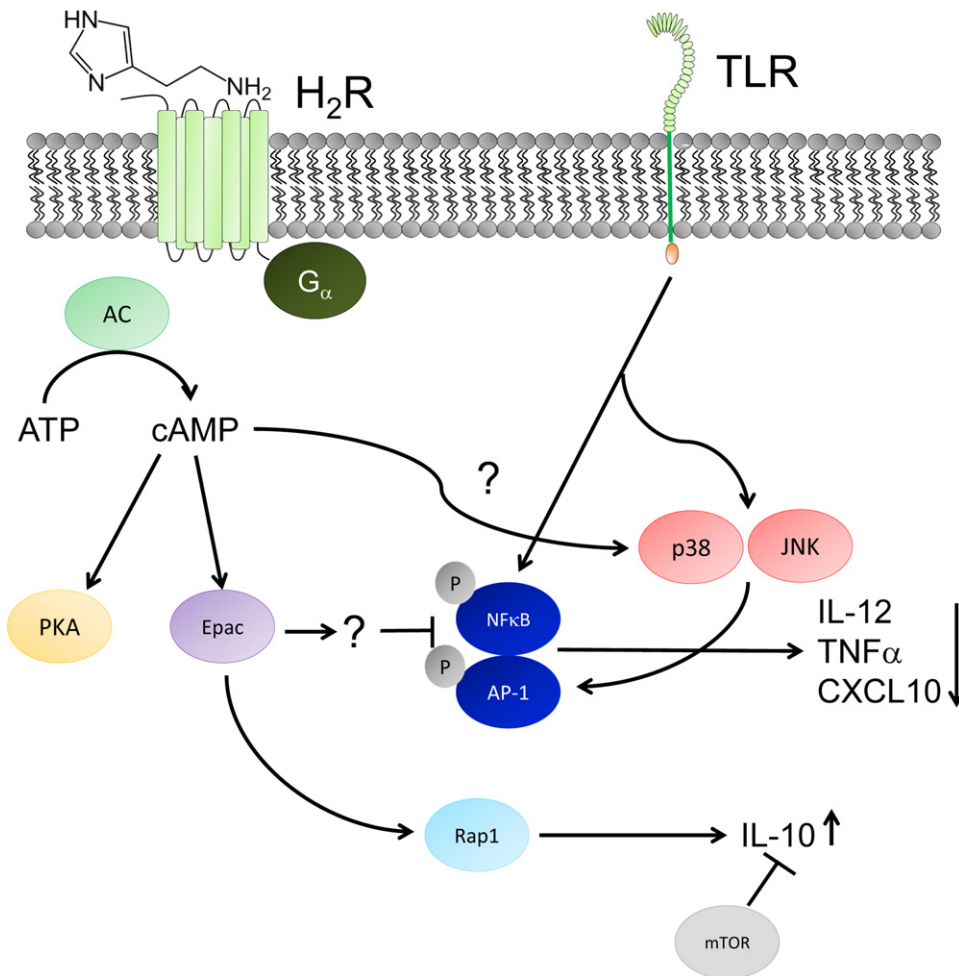


FIG 8. Schematic representation of H₂R and TLR pathway interactions. AC, Adenylate cyclase; JNK, c-Jun N-terminal kinase.

could improve mucosal immunoregulatory activity and protect against allergic sensitization and inflammatory disorders.

Key messages

- Simultaneous activation of PRRs and H₂R leads to an altered DC response to microbes.
- cAMP and Epac are critical immunomodulatory intracellular mediators for histamine-associated effects on PRR signaling.
- Expression, activity, or both of H₂R at mucosal sites could play a key role in maintaining tolerance to the microbiota and environmental antigens.

REFERENCES

- Hörmannspurger G, Clavel T, Haller D. Gut matters: Microbe-host interactions in allergic diseases. *J Allergy Clin Immunol* 2012;129:1452-9.
- O'Mahony L, Akdis M, Cramer R, Akdis CA. Novel immunotherapeutic approaches for allergy and asthma. *Autoimmunity* 2010;43:493-503.
- Frei R, Lauener RP, Cramer R, O'Mahony L. Microbiota and dietary interactions: an update to the hygiene hypothesis? *Allergy* 2012;67:451-61.
- Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 2009;461:1282-6.
- Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol* 2010;11:373-84.
- Iwasaki A, Medzhitov R. Regulation of adaptive immunity by the innate immune system. *Science* 2010;327:291-5.
- Konieczna P, Groeger D, Ziegler M, Frei R, Ferstl R, Shanahan F, et al. Bifidobacterium infantis 35624 administration induces Foxp3 T regulatory cells in human peripheral blood: potential role for myeloid and plasmacytoid dendritic cells. *Gut* 2012;61:354-66.
- Liew FY, Xu D, Brint EK, O'Neill LA. Negative regulation of toll-like receptor-mediated immune responses. *Nat Rev Immunol* 2005;5:446-58.
- Meijer K, de Vos P, Priebe MG. Butyrate and other short-chain fatty acids as modulators of immunity: what relevance for health? *Curr Opin Clin Nutr Metab Care* 2010;13:715-21.
- Jutel M, Akdis M, Akdis CA. Histamine, histamine receptors and their role in immune pathology. *Clin Exp Allergy* 2009;39:1786-800.
- Ferstl R, Akdis CA, O'Mahony L. Histamine regulation of innate and adaptive immunity. *Front Biosci* 2012;17:40-53.
- Coton M, Romano A, Spano G, Ziegler K, Vetrana C, Desmarais C, et al. Occurrence of biogenic amine-forming lactic acid bacteria in wine and cider. *Food Micro* 2010;27:1078-85.
- O'Mahony L, Akdis M, Akdis CA. Regulation of the immune response and inflammation by histamine and histamine receptors. *J Allergy Clin Immunol* 2011;128:1153-62.
- Lever A, Mackenzie I. Sepsis: definition, epidemiology, and diagnosis. *BMJ* 2007;335:879-83.
- Roduit C, Wohlgensinger J, Frei R, Bitter S, Bieli C, Loeliger S, et al. Prenatal animal contact and gene expression of innate immunity receptors at birth are associated with atopic dermatitis. *J Allergy Clin Immunol* 2011;127:179-85.

16. Vanbervliet B, Akdis M, Vocanson M, Rozières A, Benetière J, Rouzaire P, et al. Histamine receptor H1 signaling on dendritic cells plays a key role in the IFN- γ /IL-17 balance in T cell-mediated skin inflammation. *Allergy Clin Immunol* 2011;127:943-53.
17. Mazzoni A, Young HA, Spitzer JH, Visintin A, Segal DM. Histamine regulates cytokine production in maturing dendritic cells, resulting in altered T cell polarization. *J Clin Invest* 2001;108:1865-73.
18. Meiler F, Zumkehr J, Klunker S, Rückert B, Akdis CA, Akdis M. In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure. *J Exp Med* 2008;205:2887-98.
19. Novak N, Mete N, Bussmann C, Maintz L, Bieber T, Akdis M, et al. Early suppression of basophil activation during allergen-specific immunotherapy by histamine receptor 2. *J Allergy Clin Immunol* 2012;130:1153-8.
20. Aronoff DM, Canetti C, Serezani CH, Luo M, Peters-Golden M. Cutting edge: macrophage inhibition by cyclic AMP (cAMP): differential roles of protein kinase A and exchange protein directly activated by cAMP-1. *J Immunol* 2005;174:595-9.
21. Shirshev SV. Role of Epac proteins in mechanisms of cAMP-dependent immunoregulation. *Biochemistry (Mosc)* 2011;76:981-98.
22. Chen K, Xiang Y, Yao X, Liu Y, Gong W, Yoshimura T, Wang JM. The active contribution of Toll-like receptors to allergic airway inflammation. *Int Immunopharmacol* 2011;11:1391-8.
23. Reece P, Thanendran A, Crawford L, Tulic MK, Thabane L, Prescott SL, et al. Maternal allergy modulates cord blood hematopoietic progenitor Toll-like receptor expression and function. *J Allergy Clin Immunol* 2011;127:447-53.
24. Wood LG, Simpson JL, Wark PA, Powell H, Gibson PG. Characterization of innate immune signalling receptors in virus-induced acute asthma. *Clin Exp Allergy* 2011;41:640-8.
25. Metz M, Doyle E, Bindslev-Jensen C, Watanabe T, Zuberbier T, Maurer M. Effects of antihistamines on innate immune responses to severe bacterial infection in mice. *Int Arch Allergy Immunol* 2011;155:355-60.
26. Handley SA, Dube PH, Miller VL. Histamine signaling through the H(2) receptor in the Peyer's patch is important for controlling *Yersinia enterocolitica* infection. *Proc Natl Acad Sci U S A* 2006;103:9268-73.
27. Beghdadi W, Porcherie A, Schneider BS, Dubayle D, Peronet R, Huerre M, et al. Inhibition of histamine-mediated signaling confers significant protection against severe malaria in mouse models of disease. *J Exp Med* 2008;205:395-408.
28. Thomas CM, Hong T, van Pijkeren JP, Hemarajata P, Trinh DV, Hu W, et al. Histamine derived from probiotic *Lactobacillus reuteri* suppresses TNF via modulation of PKA and ERK signaling. *PLoS One* 2012;7:e31951.
29. Teuscher C, Poynter ME, Offner H, Zamora A, Watanabe T, Fillmore PD, et al. Attenuation of Th1 effector cell responses and susceptibility to experimental allergic encephalomyelitis in histamine H2 receptor knockout mice is due to dysregulation of cytokine production by antigen-presenting cells. *Am J Pathol* 2004;164:883-92.

METHODS

Quantitative real-time PCR

Total RNA was isolated with the RNeasy Plus Mini Kit (Qiagen, Venlo, Netherlands). RNA was retranscribed to cDNA using Fermentas reagents (St Leon-Rot, Germany). SYBR Green solution (Qiagen) and the suitable primer pairs (Microsynth) were used to quantify gene expression: elongation factor 1 α (EF1 α), 5'-CTGAACCATCCAGGCCAAAT-3' and 5'-GCCGTGTGGCAATCCAAT-3'; IFN- α , 5'-CCTGATGAAGGAGGAC TCCATT-3' and 5'-AAAAAGGTGAGCTGGCATACG-3'; H₁R, 5'- TCTC GAACGGACTCAGATACCA-3' and 5'- CCTGTGTTAGACCACTCC TCAA-3'; H₂R, 5'- GCTGGGCTATGCCAACTCA-3' and 5'- GGTGCG GAAGTCTCTGTTTCAG-3'; H₄R, 5'- GGGTCTTGAAGATTGTTACT CTGATG-3' and 5'- CTAGAATCATTTGGCCATTCACT-3'; TNF- α , 5'-ATGTTGTAGCAAACCTCAAGCT-3' and 5'-TTGGCCAGGAGGG CATT-3'; IL-12p35, 5'-TTCACCACTCCCAAACCTGC-3' and 5'-GAG GCCAGGCAACTCCCATTTAG-3'; IL-12p40, 5'-CGGTCATCTGCCGCA AA-3' and 5'-CCCATTTCGCTCCAAGATGAG-3'; IL-10, 5'-GTGATGCC CCAAGCTGAGA-3' and 5'-CACGGCCTTGCTCTTGTGTTT-3'; CXCL-9, 5'-TGCAAGGAACCCCAAGTAGTA-3' and 5'-GGCAAATTGTTTAAGG TCTTTCAAG-3'; and CXCL-10, 5'-TACGCTGTACCTGCATCAGCAT-3' and 5'- CTCAACACGTGGACAAAATTGG-3'.

To quantify bacterial histidine decarboxylase (HDC) gene expression, the following primer pair was used: 5'-GAT GGT ATT GTT TCK TAT GA-3' and 5'-CAA ACA CCA GCA TCT TC-3' and visualized by means of agarose gel electrophoresis, as previously described.^{E1}

Murine experiments

Wild-type BALB/c mice were obtained from Charles River Laboratories (Sulzfeld, Germany), and H₂R-deficient animals were provided by Professor

Takeshi Watanabe, Osaka, Japan.^{E2} Wild-type and knockout mice were co-housed at the AO Research Institute (Davos, Switzerland) in individually ventilated cages for the duration of the study, and all experimental procedures were carried out in accordance with Swiss law. *L. rharnosus* was grown in MRS medium (Merck, VWR International, Dietikon, Switzerland) containing 0.05% cysteine (Sigma) and 0.2% histidine (Chemie Brunschwig, Basel, Switzerland) under anaerobic conditions. Viable cells (1×10^9) were administered to each mouse daily for 9 days by means of oral gavage. Peyer patch single-cell suspensions were generated with GentleMACS tubes, according to the manufacturer's instructions (Miltenyi Biotec). Peyer patch cells were stimulated with *P. aeruginosa* lysates or PMA/ionomycin for 24 hours, and cytokine secretion was quantified with the BioPlex Multiplex Suspension Array System.

Statistical analyses

GraphPad Prism 5 software (GraphPad Software, La Jolla, Calif) was used for statistical analyses. Means plus SEMs are illustrated, and paired *t* tests (*in vitro* assays) or unpaired *t* tests (murine study) were used to calculate significance. A *P* value of less than .05 was considered significant.

REFERENCES

- E1. Coton E, Coton M. Multiplex PCR for colony direct detection of Gram-positive histamine- and tyramine-producing bacteria. *J Microbiol Methods* 2005;63: 296-304.
- E2. Kobayashi T, Tonai S, Ishihara Koga R, Okabe S, Watanabe T. Abnormal functional and morphological regulation of the gastric mucosa in histamine H2 receptor-deficient mice. *J Clin Invest* 2000;105:1741-9.

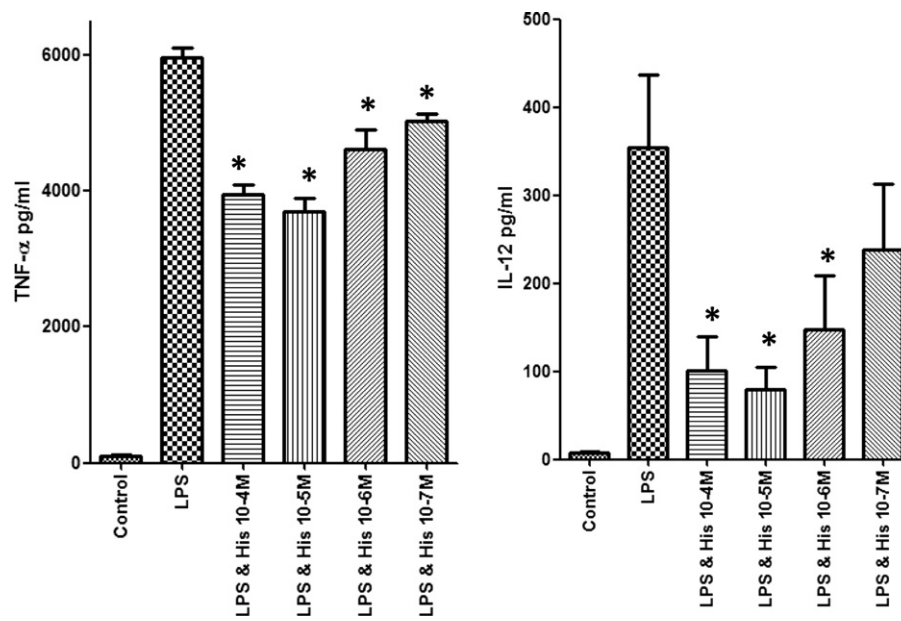


FIG E1. Histamine dose response for LPS-stimulated MDDCs. LPS-stimulated TNF- α secretion and IL-12 secretion were both significantly suppressed by histamine at 1×10^{-4} mol/L, 1×10^{-5} mol/L, and 1×10^{-6} mol/L, whereas suppression was no longer significant for IL-12 at 1×10^{-7} mol/L. Results are represented as means $\pm$ SEs ($n = 4$ donors). * $P < .05$.

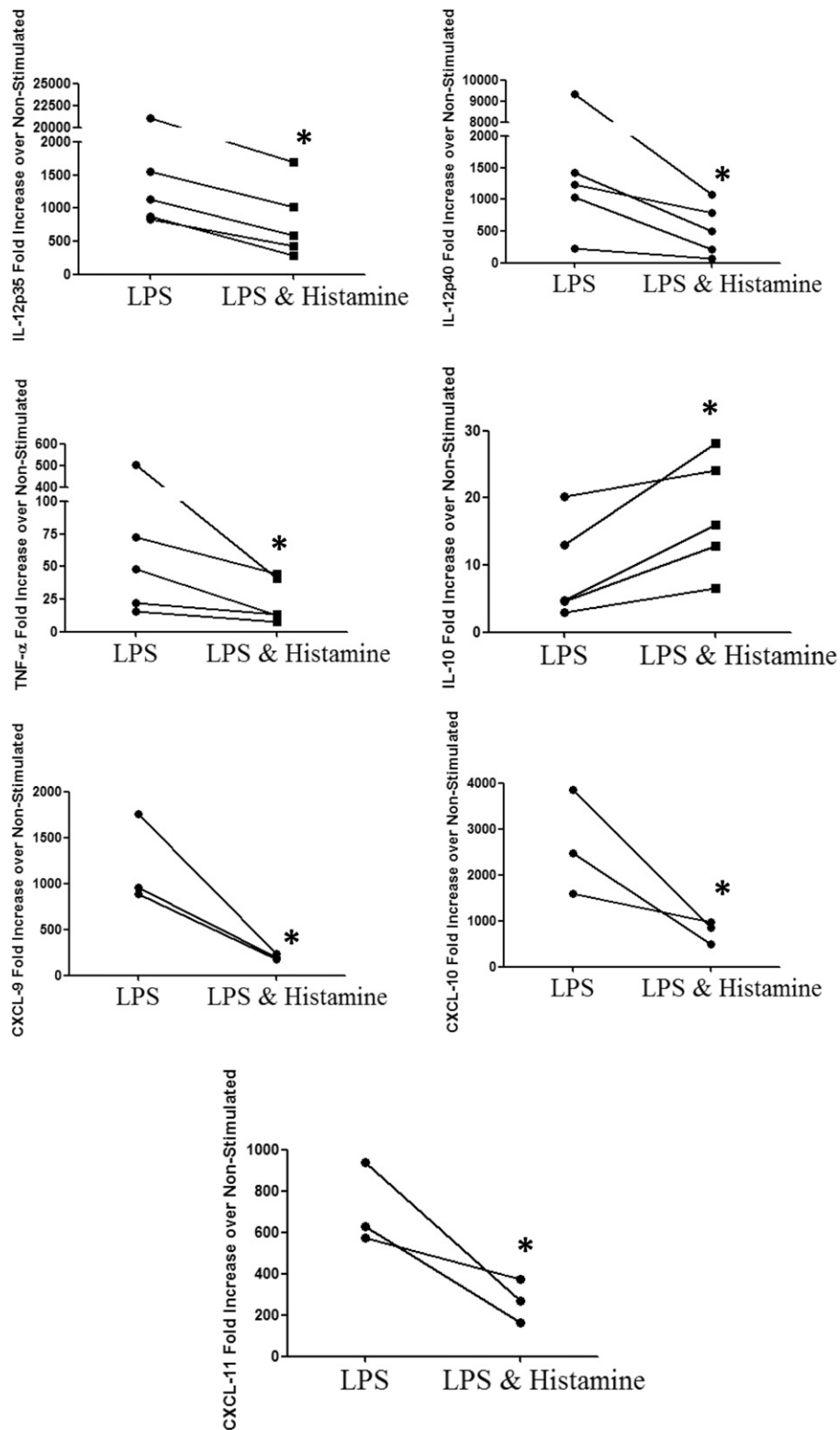


FIG E2. Histamine alters cytokine gene expression from LPS-stimulated MDDCs. LPS-stimulated IL-12p35, IL-12p40, TNF- α , CXCL9, CXCL10, and CXCL11 gene expression is significantly reduced by histamine, and IL-10 gene expression is increased 4 hours after stimulation. Each line represents an individual donor. * $P < .05$.

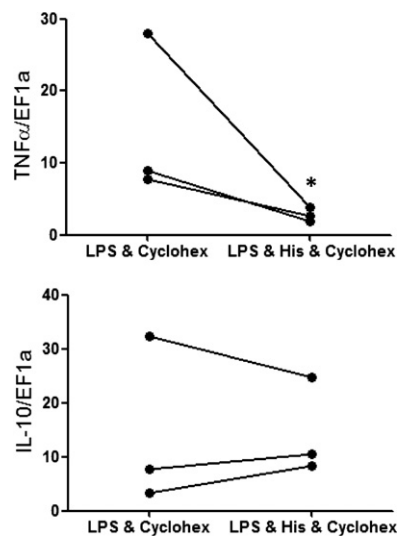


FIG E3. Modification of TNF- α , but not IL-10, gene expression does not require new protein synthesis. Histamine suppression of LPS-stimulated TNF- α gene expression was unaltered by cycloheximide. However, IL-10 gene expression did not increase when cycloheximide was present. Each *line* represents an individual donor. *EF1 α* , Elongation factor 1 α . * $P < .05$.

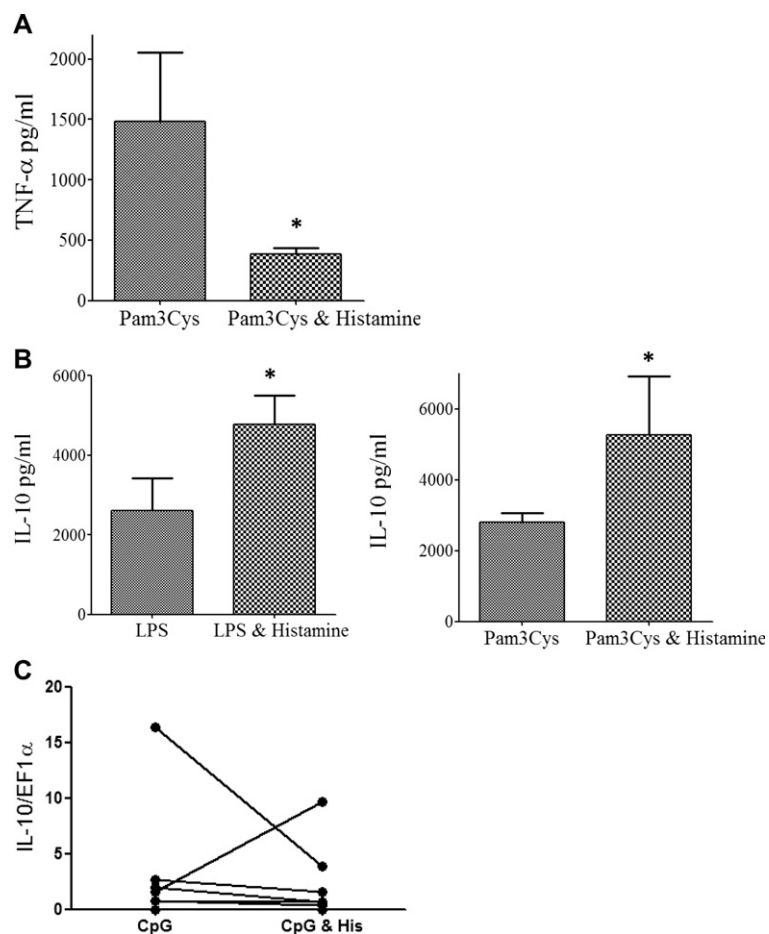


FIG E4. Histamine alters mDC cytokine secretion. **A** and **B**, Histamine suppression of Pam3Cys-stimulated TNF- α secretion by mDCs (Fig E4, **A**) was associated with enhanced secretion of IL-10 (Fig E4, **B**). **C**, In contrast, histamine did not alter CpG-induced IL-10 gene expression in pDCs. *EF1 α* , Elongation factor 1 α . * $P < .05$.

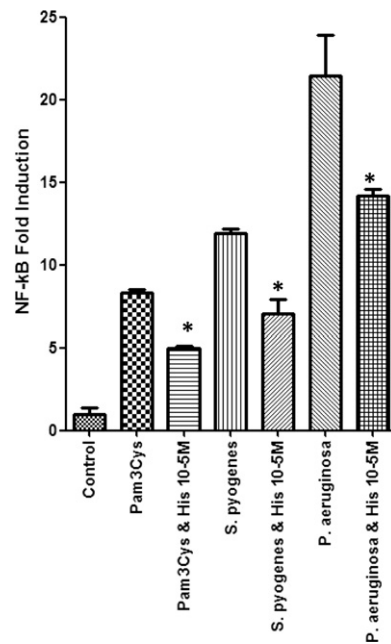


FIG E5. Histamine suppresses NF-κB/AP-1 activation. Histamine (1×10^{-5} mol/L) significantly reduces NF-κB/AP-1 activation in response to Pam3Cys, gram-positive bacteria (*Streptococcus pyogenes*), and gram-negative bacteria (*P. aeruginosa*). Results are represented as means $\pm$ SEs (n = 4 replicates per condition). * $P < .05$.

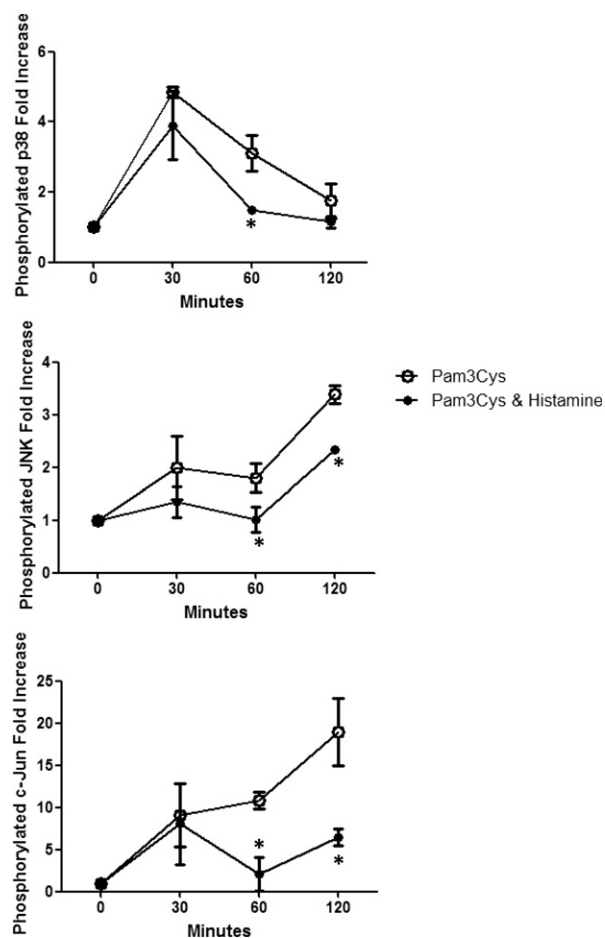


FIG E6. Histamine suppresses Pam3Cys-induced AP-1 activation. Phosphorylation of p38 and c-Jun N-terminal kinase (*JNK*) mitogen-activated protein kinase were significantly suppressed by histamine in Pam3Cys-stimulated MDDCs, as was phosphorylation of c-Jun. * $P < .05$.

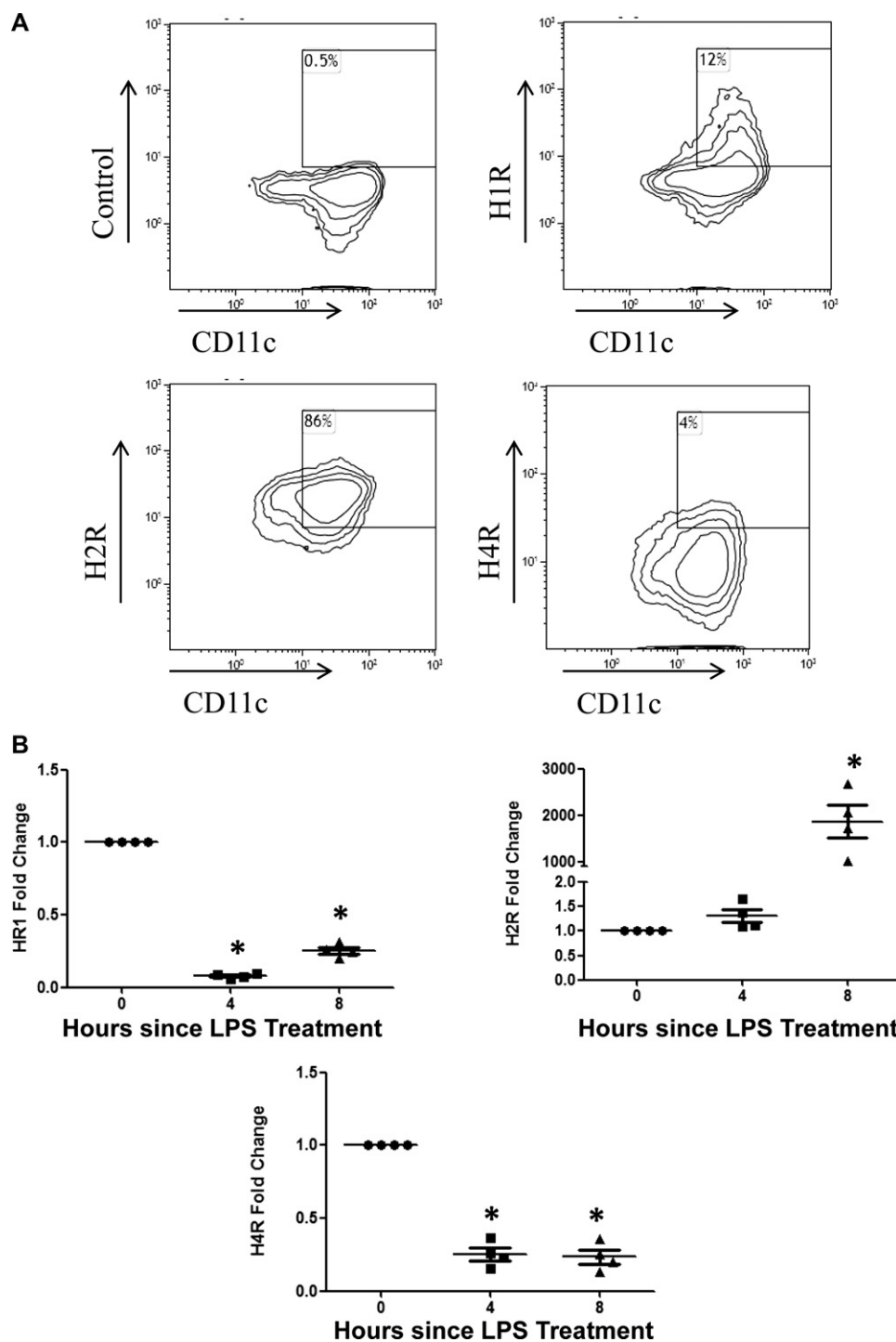


FIG E7. MDDCs express H₁R, H₂R, and H₄R. **A**, MDDC expression of histamine receptors was determined by using flow cytometry. A greater percentage of MDDCs expressed H₂R compared with H₁R or H₄R. **B**, After stimulation with LPS for 4 or 8 hours, H₁R and H₄R gene expression was significantly decreased, whereas H₂R gene expression was significantly increased at the 8-hour time point. **P* < .05.

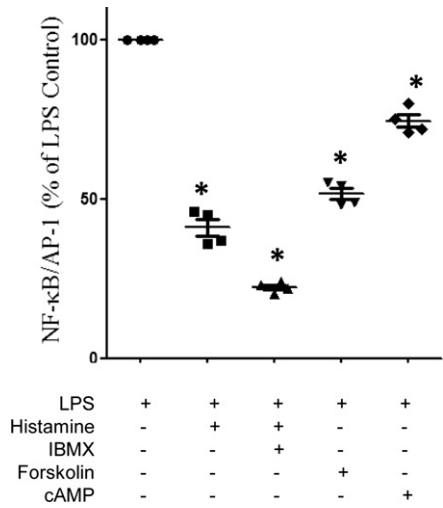


FIG E8. H₂R suppression of NF-κB/AP-1 is cAMP dependent. Histamine-associated suppression of LPS-induced NF-κB/AP-1 activation was replicated by the cAMP inducer forskolin or exogenous cAMP and was improved by the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX). The PKA inhibitor H-89 had no effect. **P* < .05.

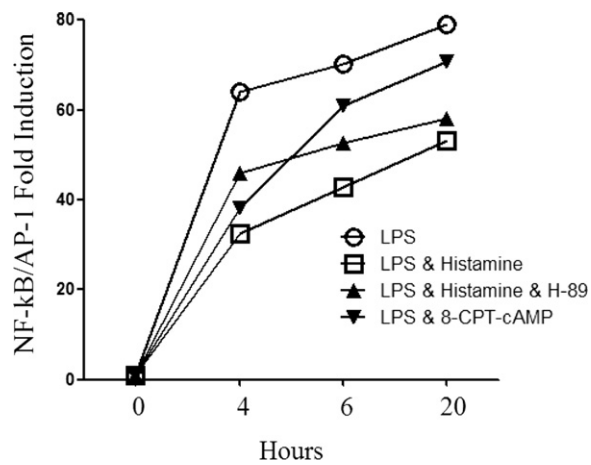


FIG E9. NF- κ B/AP-1 is associated with Epac, but not PKA, activity. Histamine suppresses NF- κ B/AP-1 activation in THP-1 cells at 4, 6, and 20 hours after LPS stimulation. The PKA inhibitor H-89 did not significantly block the suppressive effect of histamine on NF- κ B/AP-1 activity, whereas stimulation with the Epac agonist 8-CPT-cAMP replicated the effects of histamine.

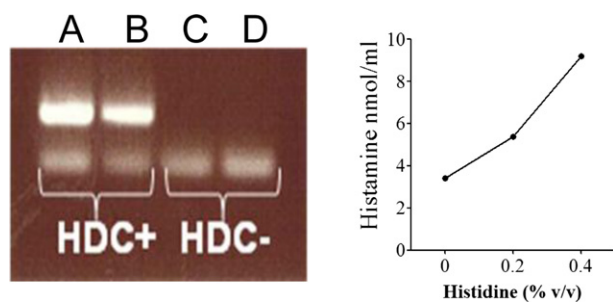


FIG E10. *L. rhamnosus* secretes histamine. *L. rhamnosus* expresses histidine decarboxylase (HDC; lanes A and B), whereas an unrelated *Bifidobacterium longum* strain does not express HDC (lanes C and D). *L. rhamnosus* also secretes histamine when histidine is present in the culture medium.

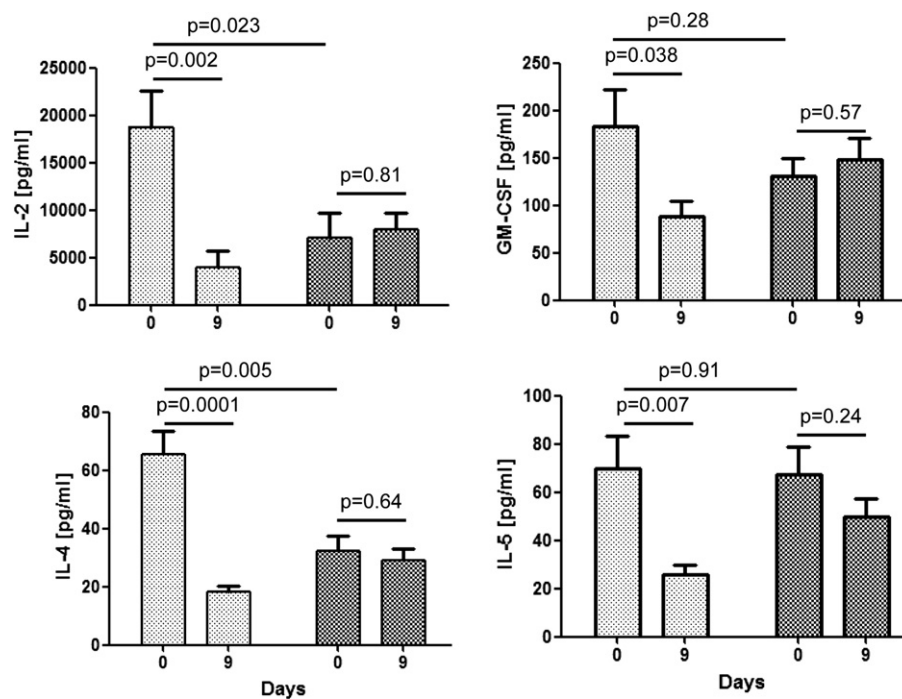


FIG E11. Differential effects of *L. rhamnosus* administration to wild-type and H₂R knockout mice. Oral administration of *L. rhamnosus* to wild-type animals (n = 10) significantly reduces PMA/ionomycin-stimulated Peyer patch cytokine secretion, which was not observed in H₂R knockout animals (n = 8). Results are expressed as means plus SEMs before (day 0) and after (day 9) feeding.