Nitric Oxide Mediates T Cell Cytokine Production and Signal Transduction in Histidine Decarboxylase Knockout Mice

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Histamine is a key regulator of the immune system. Several lines of evidence suggest the role of histamine in T cell activation and accelerated Th1 immune response is a hallmark of histidine decarboxylase knockout (HDC-KO) mice, with a complete lack of endogenously produced histamine. According to our previous work, T lymphocytes produce NO upon activation, and NO is necessary for effective T cell activation. To study the role of histamine in T cell activation, we investigated cytokine production and T cell signal transduction in HDC-KO and wild-type (WT) mice. In the absence of histamine, an elevated IFN-γ mRNA and protein levels of splenocytes \( (p < 0.001; p = 0.001, \text{respectively}) \) were associated with a markedly increased \((2.5\text{-fold}, p = 0.0009)\) NO production, compared with WT animals. Furthermore, histamine treatment decreased the NO production of splenocytes from both WT and HDC-KO mice \((p = 0.001; p = 0.0004, \text{respectively})\). NO precursor \((Z)-1-[2-(2-aminoethyl)-N-(2-ammonioethyl) amino] \text{diazen-1-ium-1,2-diolato-diethylenetriamine} \) elicited IFN-γ production \((p = 0.0002)\), whereas NO synthase inhibitors N\(^\text{G}\)-monomethyl-L-arginine and nitronidazole both inhibited IFN-γ production \((p = 0.002 \text{ and } p = 0.01, \text{respectively})\), suggesting the role of NO in regulating IFN-γ synthesis. Cytoplasmic Ca\(^{2+}\) concentration of unstimulated T cells was increased in the HDC-KO mice \((p = 0.002)\), whereas T cell activation-induced Δ Ca\(^{2+}\)-signal was similar in both HDC-KO and WT animals. Our present data indicate that, in addition to its direct effects on T lymphocyte function, histamine regulates cytokine production and T cell signal transduction through regulating NO production. *The Journal of Immunology*, 2007, 179: 6613–6619.

Engagement of TCR leads to the activation of multiple protein tyrosine and serine kinases, resulting in the phosphorylation of intracellular substrates. The subsequent hydrolysis of phospholipids and elevation of cytoplasmic Ca\(^{2+}\) ultimately leads to clonal expansion of Ag-specific T cells \((6)\). Once activated, CD4 T cells proliferate and differentiate into two main subsets of primary effector cells, Th1 or Th2 cells, characterized by their specific cytokines and IFN-γ \((8)\). Th2 cells, through the expression of IL-4, IL-5, and IL-13, induce IgE production by B cells and eosinophil-mediated mast-cell-mediated immune responses, and orchestrate the defense against extracellular parasites \((9)\). Th2 cells have a central role in driving the immune response in asthma and atopic diseases \((10)\). The Th1/Th2 balance is therefore considered to be pivotal in chronic inflammatory diseases. Recently, Th17 cells have been identified as cells induced by IL-6 and TGF-β and expanded by IL-23 \((11)\). Th17 cells have been strongly implicated as well in allergic diseases \((12)\). Histamine selectively enhances the secretion of Th2 cytokines, such as IL-4, IL-5, IL-10, and IL-13, and inhibits the production of Th1 cytokines IL-2 and IFN-γ \((13)\) and monokine IL-12 \((10, 14)\). The crucial role of histamine in the early events of the pathogenesis of atopic asthma is associated with the increased production of Th2 and decreased production of Th1 cytokines \((10)\).
Crosslinking of the TCR has been associated with elevation of the cytosolic Ca\(^{2+}\), reactive oxygen intermediate (ROI), NO production, and mitochondrial hyperpolarization (15). In contrast to histamine, NO selectively enhances Th1 cell proliferation (16) and represents an additional signal for the induction of T cell subset response (17). Although there were several pharmacological approaches to study the biological role of histamine in allergic and autoimmune diseases, its exact role in immune regulation is far from being uncovered. It has been shown that both histamine and NO can modulate the cytokine network in multiple ways, however, the possible role of NO in the immunoregulatory functions of histamine is not known (18). In our present study, we investigated the effect of genetically induced histamine deficiency on T lymphocyte cytokine expression and T cell activation, using HDC gene knock-out (HDC-KO) and congenic wild-type (WT) mice. Our results indicate that histamine deficiency profoundly alters T lymphocyte cytokine expression and T cell activation. Furthermore, our data show that, in addition to its direct effects on T cells, histamine modulates immune responses through regulating NO production.

Materials and Methods

Animals

The strategy to generate HDC-KO mice has been described previously (19). HDC\(^{+/−}\) CD1 mice were backcrossed onto the BALB/c background over nine generations. Female mice were 6–8 wk of age at the beginning of the sensitization. All mice were maintained on a histamine-free diet (Altromin). The WT and HDC-KO mice were littermates of HDC\(^{+/−}\) × HDC\(^{+/−}\) crosses. The study was approved by an Institutional Review Committee. In some experiments, WT and HDC-KO mice were injected with CFA and splenocytes were isolated 9 days later. Splenocytes were resuspended at 10⁶ cells/ml in RPMI 1640 medium supplemented with 1% FCS, 2 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml gentamicin in 12-well plates at 37°C in a humidified atmosphere with 5% CO\(_2\).

Cell viability assays

Apoptosis was monitored by observing cell shrinkage and nuclear fragmentation and was quantified by flow cytometry after concurrent staining with fluorescein-conjugated annexin V [ Annexin V-FITC; R & D Systems; fluorescence channel 1 (FL-1)] and propidium iodide (FL-2), as described earlier (15).

RT-PCR

Total RNA was isolated from spleens of CFA-injected HDC KO and WT mice using TRI-reagent (Sigma-Aldrich). First strand cDNA was produced using random hexamers (Promega). In the RT-PCR, the following primers were used: mouse IFN-γ sense: CCT CAG ACT CTT TGA AGT CT and antisense: CAG CGA CTC TTC TCT CTC TT, (annealing temperature was 54°C, 35 cycles) and mouse GAPDH sense: CTG GTG CTG AGT and antisense: CAG TCT TCT GAG TGG CAG TG (57°C, 30 cycles). The PCR were conducted using a Perkin-Elmer thermal cycler. PCR products were produced on 3% agarose gels.

Quantitative RT-PCR

T cells were isolated by using magnetic beads (negative selection) (Miltenyi Biotech), total RNA was extracted from T cells using RNeasy Mini Kit (Qiagen). First strand cDNA was produced using random hexamers (Promega). Relative quantification of neuronal NO synthase (NOS (nNOS)), inducible NOS (iNOS), and endothelial NOS (eNOS) mRNAs was performed with a TaqMan real-time RT-PCR assay on an ABI Prism 7000 Sequence Detector (Applied Biosystems) using standard protocols. In brief, 2 μl of cDNA in water was added to 12.5 μl of the 2X PCR mastermix (Applied Biosystems), primers, and probe in 1.3 μl and water to 25 μl. The reactions were amplified for 15 s at 95°C and 1 min at 60°C for 45 cycles followed by the thermal denaturation protocol. All reactions were run in duplicate and included no template and no reverse transcription controls for each gene. Analyses of real-time quantitative PCR data were performed using the comparative threshold cycle method. The relative amount of mRNA was referred to the one of hypoxanthine phosphoribosyltransferase.

ELISPOT

Splenocytes were plated in duplicates in 96-well plates (Multiscreen; Millipore) (10⁶ cells/plate) precoated with an anti-IFN-γ, anti-IL-4, or anti-IL-5 capture mAb (mouse IFN-γ, IL-4, and IL-5 Duoset; R & D Systems), blocked in the presence of 1% FCS-containing PBS for 1 h at 37°C, and were stimulated with 2 μg/ml Con-A (Sigma-Aldrich) for 24 h at 37°C, 5% CO\(_2\). After washing with PBS-Tween 20 ten times, the cells were incubated with biotinylated anti-IFN-γ, anti-IL-4, or anti-IL-4 detection mAb (mouse IFN-γ, IL-4, and IL-10 Duoset, R&D Systems) for 2 h followed by 10 washes and incubation with streptavidin-HRP conjugate for 1 h, and then, after ten further washes by the chromogenic substrate (aminoethyl carbazole, Sigma-Aldrich and H₂O₂) for 20 min, the plates were washed with distilled water. The number of IFN-γ, IL-4, or IL-10 producing spot forming cells was counted by ELISPOT reader (CTI). In some experiments, ELISPOT assays were performed in the presence of NO donor (Z)-1-[(2-aminoethyl)-N-(2-ammonioethyl)amino]di-1-imid-1,2-diolate diethylaminoethane (NOC-18) or NOS inhibitor N⁴-nomonomethyl-L-arginine (Molecular Probes), or nitroimidazole (Calbiochem).

Measurement of intracellular NO levels

Production of NO was assessed by using 4-aminomethyl-N⁴-nitro-2,3-difluoro-7,8-difluoro-7-dimethylaminonaphthalene-2-carboxylic acid (DAF-FM; Molecular Probes) (15, 20). DAF-FM passively diffuses across cellular membranes; once inside cells, it is deacetylated by intracellular esterases and caged in the cell. DAF-FM is essentially nonfluorescent until it reacts with NO to form a fluorescent benzotriazole. Measurement of NO was calibrated by incubating splenocytes with NO donors NOC-18 (60 μM to 1.8 mM). Following testing, 1–3 μM dye concentration was used in incubation buffer. After 10 min to 3 h, maximum NO sensitivity was achieved by loading cells with 1 μM DAF-FM for 2 h in 37°C. NO production within T cell subsets were concurrently analyzed by parallel staining with DAF-FM and PE conjugated mAb UCHT1 recognizing the CD3 chain (BD Biosciences). Excitation and emission maximum of DAF-FM are 495 and 515 nm, respectively.

Serum nitrite and nitrate measurements

Serum nitrate/nitrite levels (stable in vivo markers of NO production) were measured by using High-Sensitivity Nitrite Assay kit (Molecular Probes) (21).

Measurement of cytoplasmic calcium level and mitochondrial mass

Cytoplasmic calcium levels ([Ca\(^{2+}\)]\(_{cyt}\)) were assessed by loading the cells with 1 μM Fluo-3-acetoxymethyl ester (excitation, 506 nm; emission, 526 nm recorded in FL-1; Molecular Probes) (15, 20). After entering the cell, acetoxymethyl ester hydrolysis occurs and, thereafter, the dye is trapped in the cytosol. Fluo-3 elicits a large increase of fluorescence intensity on binding calcium. To investigate early Ca\(^{2+}\) fluxes, samples were incubated by Fluoro-3 and stimulated with Con-A 2 μg/ml, whereas Fluo-3 fluorescence of CD3\(^{+}\) T cells was continuously recorded by flow cytometry. In some experiments, cell permeable BAPTA-AM (Molecular Probes) was used to chelate intracellular Ca\(^{2+}\). Samples were analyzed using a FACSCalibur flow cytometer (BD Biosciences) equipped with 20-nW argon (emission at 488 nm) and 16-mW helium-neon lasers (emission at 634 nm). Dead cells and debris were excluded from the analysis by electronic gating of forward and side scatter measurements. Each measurement was conducted on 10,000 cells.

Flow cytometric analysis of mitochondrial mass and ROI production

Mitochondrial mass was monitored by staining with 50 nM nonyl acridine orange (NAO, excitation, 490 nm; emission, 540 nm recorded in FL-1) (15, 20). Fluorescent probe was obtained from Molecular Probes. ROI was monitored by 1 μM hydroethidine (Molecular Probes). Fluorescence emission from oxidized hydroethidine was detected at a wavelength of 580 nm. Dead cells and debris were excluded from the analysis by electronic gating of forward- and side scatter measurements. Each measurement was conducted on 10,000 cells.

Intracellular flow cytometry

Splenocytes from HDC-KO and WT mice were stimulated with 2 μg/ml ConA for 48 h, treated with GolgiPlug Protein Transport Inhibitor (contains brefeldin A) (BD Biosciences) and were incubated for 5 h at 37°C in a humidified CO\(_2\) incubator. Then 0.5 μg/ml fluorochrome-conjugated mAb specific for cell surface Ags (CD3-PE, CD4-PerCP, CD8-PerCP, CD45-PerCP, or CD25-PerCP-CY5, all from BD Biosciences) was added and incubated for 30 min at 4°C in the dark. Cells were washed in PBS, and...
then fixed and permeabilized by using Fixation/Permeabilization solution (BD Biosciences) for 20 min. at 4°C. Cells were washed two times in Perm/Wash buffer (BD Biosciences), then incubated 0.5 μg/ml FITC-conjugated anti-IFN-γ Ab (BD Biosciences) for 30 min at 4°C in the dark. Samples were washed in PBS and analyzed by three or four color analysis on a FACSCalibur flow cytometer (BD Biosciences) using CellQuest software version 3.1.

Mass spectrometry

One hundred milliliters of cold methanol containing 2 mM stable isotope-labeled (U-15C6, 98%; U-D5; U-15N3) L-histidine (Cambridge Isotope Laboratories) was added to 5 ml serum, and then samples were filtrated in a Millipore Multiscreen Solvent filter plate. The filtrates were evaporated to dryness under nitrogen, and 70 ml 3 N butanolic HCl was added to each sample. After esterification at 60°C for 20 min, butanol was evaporated and samples were reconstituted in 100 ml 50% acetonitrile/water, 0.02% formic acid. For quantitative analysis 10 ml of reconstituted sample was delivered to an API 2000 (Applied Biosystems) triple quadruple mass spectrometer. The intensities of ions representing nonlabeled (212.2/110.2) and isotope-labeled (226.3/123.2) histidine butyl esters were measured and the ratios of ion intensities derived from nonlabeled and labeled histidine were used for the calculation of serum histidine concentration.

Statistics

Results were analyzed by a Student’s t test or Mann-Whitney U rank sum test for nonparametric data. Changes were considered significant at p < 0.05.

Results

HDC-KO splenocytes produce increased IFN-γ at both mRNA and protein levels

Previous data from our laboratory reveal that HDC-KO mice are characterized by a Th1-biased cytokine pattern (13, 14). Because IFN-γ is a prototype of Th1 cytokines, we first studied IFN-γ production of HDC-KO and WT splenocytes. Splenocyte IFN-γ mRNA levels were measured by RT-PCR. In comparison with WT splenocytes, HDC-KO splenocytes displayed significantly higher levels of IFN-γ mRNA (p < 0.001, Fig. 1A). To further study the cytokine production of HDC-KO and WT splenocytes, IFN-γ, IL-4, and IL-10 protein levels were measured by ELISPOT method, following in vitro stimulation with 2 μg/ml Con-A. In accordance with the PCR data, the IFN-γ production of splenocytes from HDC-KO mice was higher than that of the WT animals (Fig. 1B; p < 0.0001). IL-4 and IL-10 protein levels were similar in both HDC-KO and WT splenocytes (p = 0.23 and p = 0.4, respectively, data not shown). The IFN-γ production of the CD4, CD4/CD25, CD8, and CD45 positive T cell subsets were measured by intracellular flow cytometry. IFN-γ production was similar in all these subsets of both the HDC-KO and WT T cells.

The increased IFN-γ production seems to represent a shift in the entire T cell population.

Histamine regulates NO production in T cells

NO is an important physiological regulator of T cell function (15), thus, we next studied NO synthase (NOS) expression and NO production of T lymphocytes derived from HDC-KO and WT mice. Following the isolation of T cells from splenocytes, NOS expression of the HDC-KO and WT T lymphocytes was measured by quantitative real time RT-PCR. Our data indicate the highest expression of the nNOS isoform, whereas lower levels of iNOS and eNOS isoforms were detected (Fig. 2). Although all three isoforms were expressed predominantly in the HDC-KO T cells, there was no significant difference in the expression of NOS isoforms between the WT and HDC-KO T cells.

The NO production of T lymphocytes was measured by flow cytometry. T cells from HDC-KO mice produced higher amounts of NO than those from the WT (Fig. 3A, p = 0.0009). T cell activation is associated with ROI and NO production (15). In addition to higher baseline NO production of the T lymphocytes of HDC-KO animals, T cell activation elicited accelerated NO signal (p = 0.0024, Fig. 3B). To further investigate the role of histamine in the regulation of NO production, HDC-KO and WT splenocytes were costimulated with 2 μg/ml ConA and 10⁻⁶ M histamine for 24 h, and the NO production of T cells was measured by flow cytometry. According to our data, histamine down-regulates NO production of both HDC-KO and WT T lymphocytes (Fig. 3, C and D, p = 0.0004 and p < 0.001, respectively).

Nitrite and nitrate are stable end products of NO production. Our data indicate that T cells from HDC-KO mice produce higher amounts of NO than control T cells, but a substantial amount of NO can be generated by many other cell types as well (22). To estimate total NO production, the serum nitrite and nitrate concentrations of HDC-KO and WT mice were measured. Nitrite and nitrate levels were measured both following in vivo CFA treatment and without in vivo stimulation. There was no significant difference in the nitrite and nitrate productions between the HDC-KO and WT animals in either conditions (Fig. 3E).

T cell activation-induced rapid Ca²⁺ signal is accelerated in HDC-KO T cells

Our previous data show that NO regulates the activation and signal transduction of T cells (15, 20), therefore, we next investigated the
T cell activation-induced Ca^{2+}-signal in HDC-KO and WT T lymphocytes. Activation of T cells through the TCR initiates a biphasic elevation in the cytosolic-free Ca^{2+} concentration, a rapid initial peak observed within 5–10 min, and a plateau phase lasting 4 to 48 h (6). Cytoplasmic Ca^{2+} concentration of unstimulated T cells and T cell activation-induced rapid Ca^{2+} signal are both markedly increased in HDC-KO T cells (Fig. 4A; p < 0.02; p < 0.04, respectively). Indeed, although the basal Ca^{2+} level is higher in the HDC-KO T cells, there is no difference in the stimulation-induced δ Ca^{2+} levels. T cell activation-induced sustained Ca^{2+} signal was similar both in HDC-KO and WT T lymphocytes (data not shown). To investigate the role intracellular Ca^{2+} on IFN-γ production, the effect of cell permeable intracellular Ca^{2+} chelator BAPTA-AM was studied. Con-A treatment induced IFN-γ production was inhibited by 10 μM BAPTA-AM cotreatment (Fig. 4B; p = 0.001).

NO regulates IFN-γ production

Our present data indicate that the increased NO production of HDC-KO T cells is associated with altered cytokine production and T cell signal transduction. Because NO may modulate gene transcription and cytokine production (15, 22, 23), next we investigated if NO directly regulates IFN-γ production. NO precursor NOC-18 releases NO in a dose dependent manner. Treatment of splenocytes with NO precursor NOC-18 (60 μM for 24h) increased 2 μg/ml ConA-induced IFN-γ production (Fig. 5A; 1.38 ± 0.13-fold; p = 0.0002). Six hundred μM nitronidazole and 100 μM N^G-Monomethyl-L-arginine both inhibited IFN-γ production of HDC-KO splenocytes (Fig. 5B; p = 0.01; p = 0.002, respectively). In parallel, NO production, monitored by DAF-FM fluorescence, was inhibited by >60%, following both N^G-Monomethyl-L-arginine and nitronidazole treatment. Moreover, pharmacological inhibition of NO production in HDC-KO splenocytes decreased IFN-γ synthesis, further supporting the role of NO in regulating IFN-γ production.

By contrast, treatment of splenocytes with 10^−6 M histamine did not significantly alter 2 μg/ml ConA-induced IFN-γ production (13) (data not shown). There was no significant difference in the L-histidine serum levels of the HDC-KO and WT mice (73 ±
18 μM; 64 ± 13 μM, respectively, \(p = 0.39\)). Furthermore, L-histidine treatment did not alter Con-A-induced IFN-γ production \((p = 0.3)\), measured by ELISPOT assay.

**Mitochondrial biogenesis in HDC-KO and WT T cells**

NO has recently been recognized as a key signal of mitochondrial biogenesis (15, 20). Mitochondria can take up, store, and release Ca\(^{2+}\); thus altered mitochondrial mass has a role in shaping Ca\(^{2+}\) signal in many cell types (15, 20), including T lymphocytes. Because HDC-KO T cells produce higher amounts of NO, mitochondrial mass was measured in both HDC-KO and WT T cells. Our data indicate that there is no significant difference between the mitochondrial mass of HDC-KO and WT T cells (Fig. 6; \(p = 0.1\)).
ROI production and CD3 internalization in HDC-KO and WT T cells

Because ROI production is associated with T cell activation (15), and both Ca\(^{2+}\) flux and NO production are different in HDC-KO T cells, we next investigated constitutive and activation-induced ROI production. Our data indicate that basal ROI production and 4 h Con-A stimulation-induced ROI production are similar both in the HDC-KO and the WT T cells (Fig. 7). However, the 24 h Con-A treatment-induced ROI signal was smaller in the HDC-KO T cells (Figure 7). This is in accordance with our previous data, indicating that NO regulates T cell activation-induced ROI signal (15). The T cell activation-induced CD3 internalization was similar in both HDC-KO and WT T lymphocytes (data not shown).

Discussion

Histamine is an important mediator of allergic diseases and inflammation (24). In addition, involvement of histamine in the molecular machinery of melanoma progression have been reported recently (25, 26). The development of some allergic reactions, infection, and tumors are associated with excessive histamine production. Histamine modulates the cytokine production of immunocompetent cells, including T lymphocytes, by binding to histamine receptors on their cell surface. T cells express both type 1 and type 2 histamine receptors, histamine inhibits the production of Th1 cytokines such as IL-2 and IFN-\(\gamma\) and enhances the secretion of Th2 cytokines such as IL-4, IL-5, IL-10, and IL-13. Histamine shifts the Th1/Th2 balance toward Th2, via regulation of JAK-STAT signal transduction pathway (27). Decreased production of IFN-\(\gamma\) following histamine treatment, was reported in activated human blood mononuclear cells (13, 28, 29). Our present data confirm and extend the observations of others, regarding the immunoregulatory role of histamine. In the present study, we show for the first time that T lymphocytes in vivo, in the absence of histamine, exhibit Th1-type cytokine dominance, as they produce higher levels of IFN-\(\gamma\) both at mRNA and protein levels. Our data indicate that histamine deficiency is associated with a markedly increased T cell NO production, and histamine directly regulates NO production. Thereafter, NO may contribute to the shifted cytokine profile of HDC-KO T cells.

NO is a diffusible, multifunctional, transcellular messenger that has been implicated in numerous physiological and pathological conditions (22). NO is synthetized from l-arginine by NOS. Three distinct isoforms of NOS are known, including nNOS, iNOS, and eNOS enzymes. According to our present data, mouse T cells predominantly express the nNOS isoform, while lower level of iNOS and eNOS isoforms were detected (15, 30). Although the iNOS activity depends on transcription, the eNOS and the nNOS are constitutively expressed and are activated upon elevated intracellular Ca\(^{2+}\) (30). Cytoplasmic Ca\(^{2+}\) level is higher (\(p = 0.002\)) in HDC-KO T cells which may activate eNOS and nNOS enzymes and induce NO production. According to our previous data, NO donor treatment increases both cytoplasmic Ca\(^{2+}\) concentration and T cell activation induced Ca\(^{2+}\) signal (20), which observation is in accordance with our present data. Furthermore, the NO production and the T cell activation-induced NO signal are both higher in HDC-KO T cells. Our data suggest that histamine is an additional factor that regulates NO production. This is in accordance with our previous observations, confirming an important role of NO in T cell activation.

NO promotes mitochondrial hyperpolarization, ATP depletion, and relative resistance to apoptotic stimuli in astrocytes, lymphocytes, and Jurkat cells (23, 31, 32). Low concentrations of NO may specifically inhibit cytochrome-c oxidase, leading to ATP depletion (33). NO has newly been recognized as a key signal for mitochondrial biogenesis. This operates through the cGMP-dependent peroxisome proliferator-activating receptor \(\alpha\) coactivator-1\(\alpha\), a master regulator of mitochondrial biogenesis. NO was shown to induce mitochondrial biogenesis in brown adipocytes, U937 and HeLa cells, and human lymphocytes (34). According to our present data, increased NO production of HDC-deficient mice is not associated with increased mitochondrial biogenesis.

Earlier studies (35) showed that mice deficient in iNOS developed an enhanced Th1 response, following infection with protozoa Leishmania major, suggesting a role of NO in regulating Th1/Th2 phenotypes. NO was reported to inhibit (36, 37) Th1-type responses (IL-2, IFN-\(\gamma\)) but not Th2-type responses (IL-4 and IL-10). By contrast, other findings suggest that NO preferentially promotes IFN-\(\gamma\) synthesis (38, 39) and type 1 Th cell differentiation by selective induction of IL-12R\(\beta\)2 via cGMP (16). Elevated levels of cGMP, corresponding to enhanced Th1 cell activation, were detected in CD4\(^{+}\) T cells 30 min after the cells were exposed to NO. Naive T cells express IL-12R upon TCR engagement. The expression of IL-12R appears to be transient and is down-regulated in committed memory Th1 cells, which can be maintained and expanded by other factors including IL-15 (40). NO is an additional factor for enhancing IL-12R expression and, thus, promoting type 1 cell differentiation (38). Thereafter, the regulatory role of NO on Th1/Th2 polarization appears to be concentration dependent, low NO concentrations selectively enhance the induction of Th1 cells, while higher concentrations may inhibit Th1 response. The polarization of naive T cells toward Th1 or Th2 phenotypes is mediated primarily by dendritic cells (DCs). Both mast cells and iDCs are located in the periphery, often in close proximity to each other. Both the cytokines that are expressed by DCs and the capacity of DCs to polarize naive T cells are regulated by histamine (41). Our previous data revealed that histamine has a negative effect on the Ag presentation and the adaptive immune response by altering the cytokine profile of DCs (14). Complete and long-lasting elimination of histamine in the experimental animals in vivo cannot be achieved by the currently available pharmacological inhibitors. Due to the overlapping and sometimes antagonistic function of the receptors in the presence of endogenous histamine, receptor blocking alone cannot achieve complete blockade of the histamine system. Therefore, knockout mice with defective histamine synthesis were used in our work to investigate the role of histamine in T cell signal transduction.

Both histamine and NO are well-known regulators of the immune responses. Collectively, our present data indicate that histamine can modulate the T cell-mediated immune response through regulating NO production. These data contribute to the understanding of the increasingly complex network of immune regulation essential for health and disease.

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Disclosures

The authors have no financial conflict of interest.

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