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Cutting Edge: Histamine Receptor H₄ Activation Positively Regulates In Vivo IL-4 and IFN- γ Production by Invariant NKT Cells¹

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Histamine (HA) is a biogenic amine with multiple activities in the immune system. In this study we demonstrate that histamine-free histidine decarboxylase-deficient (HDC^{-/-}) mice present a numerical and functional deficit in invariant NK T (iNKT) cells as evidenced by a drastic decrease of IL-4 and IFN- γ production. This deficiency was established both by measuring cytokine levels in the serum and intracellularly among gated iNKT cells. It resulted from the lack of HA, because a single injection of this amine into HDC^{-/-} mice sufficed to restore normal IL-4 and IFN- γ production. HA-induced functional recovery was mediated mainly through the H₄ histamine receptor (H₄R), as assessed by its abrogation after a single injection of a selective H₄R antagonist and the demonstration of a similar iNKT cell deficit in H₄R^{-/-} mice. Our findings identify a novel function of HA through its H₄R and suggest that it might become instrumental in modulating iNKT cell functions. *The Journal of Immunology*, 2009, 182: 1233–1236.

Histamine (HA)³ is one of the most versatile biogenic amines with multiple physiological functions in the CNS, the intestinal tract, and inflammatory reactions. More recently, a number of studies have established that besides its most obvious contribution to allergic reactions, HA also exerts more subtle regulatory functions influencing the orientation of the immune response, thus rekindling interest in this field of investigation (1–4). It has been assumed until lately that these immunomodulatory effects were mediated mainly through classical HA receptors of the H₁ and H₂ subtypes (5, 6). However, this explanation has since been complicated by

the identification of organic cation transporter 3, OCT3, as a means through which HA can be taken up by murine basophils and exert a negative feedback on their HA, IL-4, IL-6, and IL-13 production (7), as well as by the identification of an additional HA receptor, HA receptor subtype 4 (H₄R) expressed mainly in hematopoietic and immunocompetent cells (8, 9). The most clearly established activities of H₄R consist in the recruitment and activation of cells involved in inflammatory responses such as eosinophils, mast cells, neutrophils, conventional T lymphocytes, and dendritic cells (10–13). However, its functional expression in the immunoregulatory invariant NK T (iNKT) cells has not been investigated so far.

iNKT cells constitute a distinctive population of mature T lymphocytes positively selected by the nonpolymorphic MHC class-I-like molecule CD1d. They coexpress a highly restricted TCR repertoire composed of a single invariant V α 14J α 18 chain in mice and a V α 24J α 18 chain in humans, preferentially paired with a limited TCR V β -chain repertoire that specifically recognizes glycolipids (14–16). iNKT cells are implicated in the control of several immune responses, most likely because of their capacity to promptly produce several cytokines (14–20) such as IL-4 and IFN- γ . In the present study we demonstrate that HA participates in this functional tuning to ensure optimal IL-4- and IFN- γ production by iNKT cells.

Materials and Methods

Animals

Male C57BL/6J mice (7–9 wk old) were purchased from Janvier. Histidine decarboxylase (HDC)-deficient (HDC^{-/-}) and H₄R^{-/-} mice, backcrossed 12 and 10 times to C57BL/6J mice, respectively (21, 22), were bred in our own facilities. HDC^{-/-} mice received a histamine-low diet (SAFE Scientific Animal Food and Engineering) to avoid exogenous uptake. Animal experiments were performed according to the French institutional committee.

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³ Abbreviations used in this paper: HA, histamine; α -GalCer, α -galactosylceramide; H₄R, HA receptor subtype 4; HDC, histidine decarboxylase; iNKT, invariant NK T (cell); MNC, mononuclear cell.

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In vivo treatment

Mice received a single i.p. administration of 2 μg of α -galactosylceramide (α -GalCer; Alexis) 90 min before sacrifice. In some experiments, mice were injected i.p. 1 h before α -GalCer administration with a single dose of HA (Sigma-Aldrich) (20 mg/kg) with or without the H4R antagonist JNJ 7777120 (23) (20 mg/kg) administered i.p. 1 h before HA injection.

Cell preparation

Lymphocytes were isolated from the spleen using a homogenizer. Mononuclear cells (MNC) were separated from hepatocytes and cellular debris by way of a 35% isotonic Percoll density gradient (Amersham Biosciences). Liver and spleen MNC were depleted of RBC using red cell lysis buffer (8.3 mg/ml NH_4Cl , 1 mg/ml KHCO_3 , and 3.72 $\mu\text{g}/\text{ml}$ EDTA).

Flow cytometry

Splenocytes and liver MNC were preincubated with mAbs against Fc γ R (clone 2.4G2 culture supernatant), washed, and incubated with CD1d- α -GalCer tetramer-allophycocyanin or control tetramers, anti-CD4 PerCP-Cy-5.5, anti-TCR β -FITC, anti-IL-4-PE, anti-IFN- γ -PE, or isotype control (BD Pharmingen) as described (20). In some experiments an anti-H4R (clone Y-19; Santa Cruz Biotechnology) was used according to the manufacturer's instructions. Cells were analyzed on a FACSCanto II (BD Biosciences) flow cytometer using FACSDiva software.

Determination of cytokines

IL-4 and IFN- γ were measured by ELISA as described (20).

Statistical analysis

The nonparametric test *t* was used to calculate significance levels for all measurements. Values of $p < 0.05$ were considered statistically significant.

Results and Discussion

IL-4 and IFN- γ production by iNKT cells is decreased in HA-free HDC $^{-/-}$ mice

Prompt production of IL-4 and IFN- γ in response to TCR cross-linking constitutes a typical feature of iNKT cells. We measured these cytokines to establish whether exogenous HA participated in their modulation. To this end, we injected wild-type and histamine-free HDC $^{-/-}$ mice (deficient for the HA-forming enzyme HDC) with α -GalCer, a glycolipid widely used as a specific activator of iNKT cells, to determine its capacity to specifically activate and promptly induce these cytokines. We found that both IL-4 and IFN- γ levels generated after a single injection were significantly lower in the serum of HDC $^{-/-}$ mice than in wild-type controls (Fig. 1, *A* and *B*). This decrease could result either from a lower incidence or a functional defect of iNKT cells. Indeed, we found that CD1d/ α -GalCer tetramer $^+$ cells were effectively reduced in spleen and liver of HDC $^{-/-}$ mice, both in terms of cell counts and percentage (Fig. 1, *C–E*).

The lower iNKT cell counts in histamine-free mice do not exclude the presence of functional deficiencies in the remaining cells, promoting us to analyze cytokine production in single cells by intracellular staining. It turned out that among gated iNKT cells the percentage that was actually positive for IL-4 and IFN- γ cells after injection of α -GalCer was strikingly reduced in HDC $^{-/-}$ mice compared with controls (Fig. 2, *A* and *B*). These data clearly show that iNKT cells are both numerically and functionally impaired in histamine-deficient mice.

HA injection restores the IL-4- and IFN- γ -producing capacity of iNKT cells

To confirm the implication of HA in the cytokine-producing capacity of iNKT cells, HDC $^{-/-}$ mice were treated with HA 1 h before α -GalCer stimulation. Remarkably, a single injection of HA was sufficient to restore the seric levels of IL-4 and

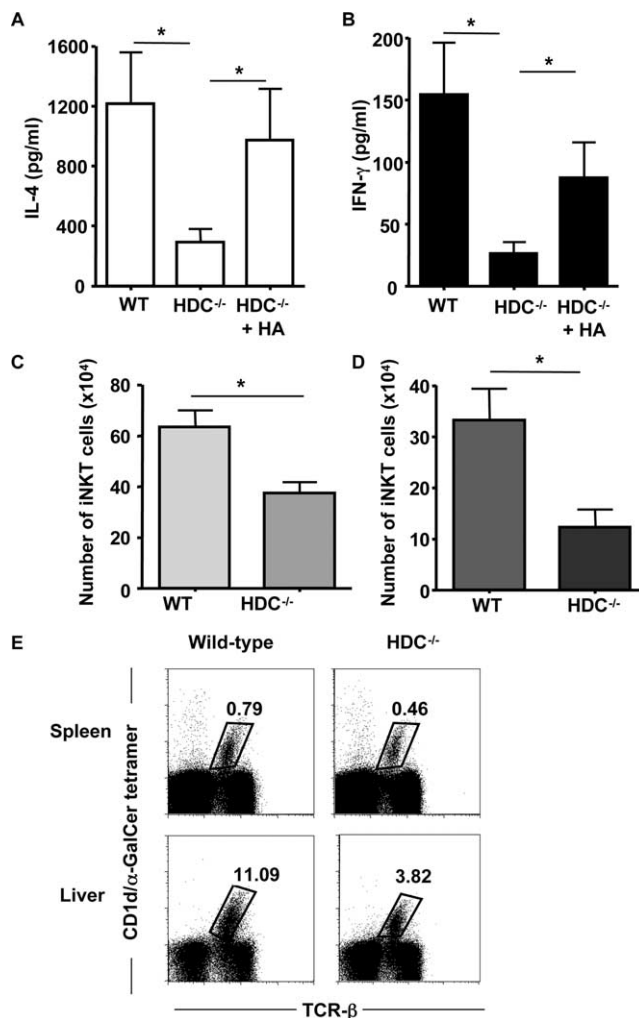


FIGURE 1. Marked decrease in cytokine production by iNKT cells in HDC $^{-/-}$ mice. Wild-type (WT) and HDC $^{-/-}$ mice were injected with α -GalCer. A group of HDC $^{-/-}$ mice were treated with HA 1 h before α -GalCer injection (HDC $^{-/-}$ + HA). Ninety minutes later, IL-4 (*A*) and IFN- γ (*B*) were measured in the serum. No cytokine was detected without α -GalCer stimulation (data not shown). The total number (*C* and *D*) and the percentage (*E*) of iNKT cells were analyzed in both spleen (*C* and *E*) and liver (*D* and *E*) from untreated wild-type or HDC $^{-/-}$ mice. Data represent the mean \pm SEM from 8–20 mice (*A* to *D*). *, $p < 0.05$. Representative FACS profiles showing the percentage of CD1d/ α -GalCer $^+$ TCR β^+ cells (*E*) in wild-type vs HDC $^{-/-}$ mice.

IFN- γ in HDC $^{-/-}$ mice (Fig. 1, *A* and *B*). Even though this treatment did not enhance the percentage or the absolute number of iNKT cells significantly (data not shown), it did increase the proportion of IL-4 $^+$ and IFN- γ^+ cells among gated iNKT lymphocytes (Fig. 2), consistent with the restored seric cytokine levels. It can therefore be concluded that HA is capable of up-regulating both IL-4 and IFN- γ production by iNKT cells activated in vivo.

Cytokine production by iNKT cells is impaired in H4 receptor-deficient mice

Knowing that histamine exerts its biological effect through four specific receptors and that the most recently discovered H4 subtype is preferentially expressed in hematopoietic cells, we used mice in which the corresponding gene had been disrupted (H4R $^{-/-}$) to assess their IL-4 and IFN- γ production following

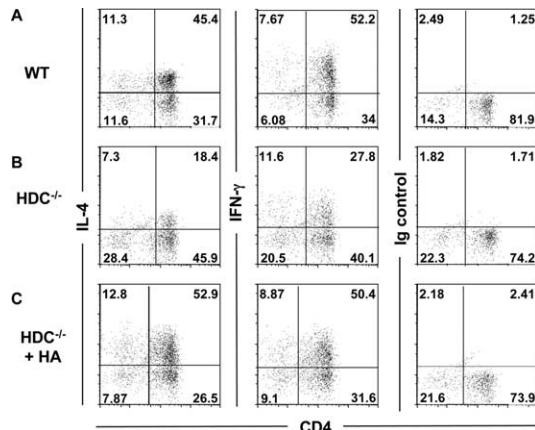


FIGURE 2. Impaired IL-4 and IFN- γ production by in vivo α -GalCer-stimulated iNKT cells from HDC^{-/-} mice. Representative FACS profiles showing the percentage of IL-4, IFN- γ , and isotype control (Ig control) positive cells among gated CD1d/ α -GalCer tetramer⁺TCR β ⁺ iNKT splenocytes from α -GalCer treated wild-type (WT) (A) or HDC^{-/-} mice (B). In the later group, HDC^{-/-} mice were treated with HA (HDC^{-/-} + HA) before α -GalCer injection (C).

α -GalCer injection. We found that H4R^{-/-} mice, which presented no significant modification in the absolute number of iNKT cells ($5.2 \times 10^5 \pm 0.9 \times 10^5$ vs $6.7 \times 10^5 \pm 1.2 \times 10^5$ splenic iNKT cells from wild-type and H4R^{-/-} mice, respectively), generated significantly fewer circulating cytokines than wild-type controls (Fig. 3, A and B), suggesting that the positive effect of HA on these biological activities was mediated through H4R activation.

In vivo treatment of HDC^{-/-} mice with a H4R antagonist abrogates the restoration of iNKT cell functions in response to HA

To prove that H4Rs were required for the up-regulation of IL-4 and IFN- γ production by iNKT cells, we blocked their binding sites with the highly selective H4 antagonist JNJ7777120 injected 1 h before HA into HDC^{-/-} mice. In this case HA failed to restore a normal IL-4 and IFN- γ production, as shown in Fig. 3, C and D. In further support of this result, we show that iNKT cells express H4R (Fig. 3E), leading us to conclude that HA modulates iNKT cell functions through this receptor subtype.

The contribution of iNKT cells to immune responses is complex because of their capacity to produce both IFN- γ and IL-4, thereby supporting Th1 or Th2 responses, respectively. We and others have reported that iNKT cells can aggravate asthma through their Th2 cytokine profile (18, 24). Similarly, HA plays a major role in atopic diseases, namely in allergic asthma, because its release in the airways is one of the typical features of this pathology that triggers a cascade of events, including airway constriction, mucus secretion, vascular leak, and recruitment of immune cells. Our present data suggest an additional means for HA to enhance the severity of asthma by promoting optimal IL-4 production by iNKT cells. Consistent with this assumption, it has recently been reported that asthmatic mice treated with the JNJ7777120 antagonist used herein develop less airway inflammation than untreated controls (11).

Taken together, our data reveal a new role of histamine through H4R activation in the functional modulation of the immunoregulatory iNKT cell population and provide addi-

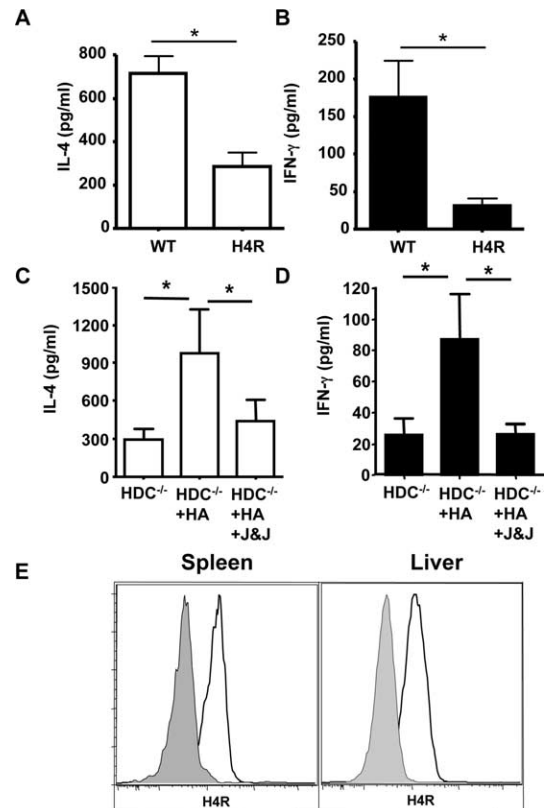


FIGURE 3. HA can modulate iNKT cell functions through H4R. A and B, Wild-type (WT) and H4R^{-/-} mice were injected with α -GalCer. Ninety minutes later, IL-4 (A) and IFN- γ (B) were measured in the serum. C and D, HDC^{-/-} mice were treated or not with the selective H4R antagonist JNJ7777120 (J&J), followed by HA and then α -GalCer injection as described in *Materials and Methods*. Ninety minutes after the last injection, IL-4 (C) and IFN- γ (D) were measured in the serum. Data represent the mean \pm SEM from 6–10 mice; *, $p < 0.05$. E, Representative FACS profiles showing the expression of H4R (empty histogram) compared with control (filled histogram) among gated CD1d/ α -GalCer⁺TCR β ⁺ splenocytes (left panel) or liver MNC (right panel) recovered from wild-type mice.

tional evidence for the complex influence of the microenvironment on iNKT cell functions.

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Disclosures

The authors have no financial conflict of interest.

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