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Neuropeptide Signaling Activates Dendritic Cell-Mediated Type 1 Immune Responses through Neurokinin-2 Receptor

Hidemitsu Kitamura,* Minoru Kobayashi,* Daiko Wakita,* and Takashi Nishimura*†

Neurokinin A (NKA), a neurotransmitter distributed in the central and peripheral nervous system, strictly controls vital responses, such as airway contraction, by intracellular signaling through neurokinin-2 receptor (NK2R). However, the function of NKA–NK2R signaling on involvement in immune responses is less-well defined. We demonstrate that NK2R-mediated neuropeptide signaling activates dendritic cell (DC)-mediated type 1 immune responses. IFN-γ stimulation significantly induced NK2R mRNA and remarkably enhanced surface protein expression levels of bone marrow-derived DCs. In addition, the DC-mediated NKA production level was significantly elevated after IFN-γ stimulation in vivo and in vitro. We found that NKA treatment induced type 1 IFN mRNA expressions in DCs. Transduction of NK2R into DCs augmented the expression level of surface MHC class II and promoted Ag-specific IL-2 production by CD4+ T cells after NKA stimulation. Furthermore, blockade of NK2R by an antagonist significantly suppressed IFN-γ production by both CD4+ T and CD8+ T cells stimulated with the Ag-loaded DCs. Finally, we confirmed that stimulation with IFN-γ or TLR3 ligand (polynosinic-polycytidylic acid) significantly induced both NK2R mRNA and surface protein expression of human PBMC-derived DCs, as well as enhanced human Tac1 mRNA, which encodes NKA and Substance P. Thus, these findings indicate that NK2R-dependent neuropeptide signaling regulates Ag-specific T cell responses via activation of DC function, suggesting that the NKA–NK2R cascade would be a promising target in chronic inflammation caused by excessive type 1-dominant immunity.


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*Division of Immunoregulation, Section of Disease Control, Institute for Genetic Medicine, Hokkaido University, Sapporo 060-0815, Japan; and †Division of ROYCE Health Bioscience, Section of Disease Control, Institute for Genetic Medicine, Hokkaido University, Sapporo 060-0815, Japan

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Address correspondence and reprint requests to Dr. Takashi Nishimura, Division of Immunoregulation, Institute for Genetic Medicine, Hokkaido University, Kita-15, Nishi-7, Kita-ku, Sapporo 060-0815, Japan. E-mail address: tak24@igm.hokudai.ac.jp

Abbreviations used in this article: 7AAD, 7-aminoactinomycin D; AHR, airway hyperresponsiveness; BALF, bronchoalveolar lavage fluid; COPD, chronic obstructive pulmonary disease; DC, dendritic cell; DT, diphtheria toxin; DTR, diphtheria toxin receptor; GR, GR 159897; hNK2R, human neurokinin-2 receptor; hTAC1, human Tac1; NKA, neurokinin A; NK2R, neurokinin-2 receptor; poly I:C, polyinosinic-polycytidylic acid; SP, substance P; Tg, transgenic.
medium containing 10% FCS in the presence of murine GM-CSF (20 ng/ml) or the culture supernatant of GM-CSF-producing CHO cells, as described previously (35). Loosely adherent clustering cells were harvested on days 6–8, and CD11c+ DCs were isolated by the IMag Cell Separation System with anti-CD11c mAb-bound beads or FACS(Aria) (BD Biosciences). The sorted CD11c+ DCs were immature (CD11c+MHC class II(−)), and the purity was >95% (data not shown). These DCs were used for the present experiments. The generated DCs were treated with murine IFN-γ (10 ng/ml), NKA (1 µM), or LPS (1 µg/ml) for 6 h and used for the evaluation of mRNA expression levels. For analysis of NK2R protein expression levels, DCs were treated with IFN-γ (10 ng/ml) for 24 h. For NKA assays, DCs were treated with IFN-γ (2, 4, or 20 ng/ml) for 24 h.

Depletion of CD11c+ population with DT in lung of CD11c-DTR Tg mice

PBS or DT (1 µg) was injected intranasally into CD11c-DTR Tg mice. After 24 h, recombinant IFN-γ (3 µg) was administered intranasally into the mice. Total cells were collected from collagenase-treated lung tissues or bronchoalveolar lavage fluid (BALF) 24 h after the administration of IFN-γ into the PBS- or DT-treated CD11c-DTR Tg mice, and the number of cells was counted. CD11c- and CD11b-expressing populations were analyzed by flow cytometry, and the number of CD11c+ cells was calculated. NKA levels in the collected BALF were measured by enzyme immunoassay 24 h after IFN-γ administration.

Transduction of NK2R gene in bone marrow-derived DCs

Mouse NK2R with FLAG cDNA was constructed by reverse transcription of total RNA from bone marrow-derived DCs with specific primers. The resulting cDNA was inserted into pMX-IRES-GFP retroviral vector obtained from Dr. T. Kitamura (University of Tokyo). The transduction of pMX-IRES-GFP (Mock) or pMX-NK2R-GFP into DCs was performed as described (36). For the following analyses, we sorted GFP+ cells from the retrovirus-infected DCs using FACS(Aria). Briefly, the DCs were harvested by pipetting and stained with anti-CD11c and 7AAD. The DCs gated by 7AAD+CD11c+GFP+ populations were sorted by FACS(Aria). Flow cytometry showed that the purity of the Mock- or NK2R-transduced DCs was >95% (data not shown). The NK2R-transduced DCs totally expressed NK2R-FLAG protein on the cell surface (data not shown).

Generation of human DCs from PBMCs

Research protocols for human subjects were approved by the Institutional Review Boards of Hokkaido University Graduate School of Medicine and the Institute for Genetic Medicine. Written informed consent was obtained from all healthy individuals. PBMCs were obtained from individuals by Ficoll-Hypaque (Amersham Bioscience, Uppsala, Sweden) gradient centrifugation after obtaining their informed consent. PBMCs (2 × 10^7 cells) were cultured with AIM-V (Invitrogen, Carlsbad, CA) in the presence of recombinant human IL-4 or IL-3 plus GM-CSF for 7 d, as described previously (37). We evaluated phenotypes of the generated human DCs. We confirmed that >60% of whole-cell populations expressed CD11c and HLA-DP, -DQ, and -DR, although >10% were found to be II° populations (data not shown). The generated DCs were left untreated (control) or were treated with IFN-γ (20 ng/ml) or poly I:C (20 ng/ml) for 24 h, and the expression levels of human NK2R (hNK2R) or human TAC1 (hTAC1) were evaluated.

PCR

Total RNA was extracted from murine and human DCs with an Isogen RNA extraction kit (Nippon Gene, Japan). First-strand cDNA was synthesized using 1 µg total RNA with the oligo deoxithymidine primer (Invitrogen), at a 1:20 ratio, with Superscript III reverse transcriptase (Invitrogen). Genes for murine NK2R (Tarc2), IL-12p40 (Ifnab), IFN-β (Ifnb), β-actin (Actb), hNK2R (Tarc2), hTAC1 (Taci), and human GAPDH (GAPDH) were amplified using a thermal cycler (LightCycler; Roche, Indianapolis, IN). The primer sequences used in this study were as follows: NK2R (forward: 5′-AATGACAACGGAGGCAAGAT-3′, reverse: 5′-AACGTGAGAACTACACTG-3′, Universal probe: #4), IL-12p40 (forward: 5′-GATTCACTCCAGGAGGACCA-3′, reverse: 5′-TTGTTACCCTGAGGACACT-3′, Universal probe: #27), IFN-α (forward: 5′-CCCTGGTGATGCGAGAAAC-3′, reverse: 5′-TACCTCACCG-3′, Probe: 5′-TACCTGGTGATGCGAGAAAC-3′), IFN-β (forward: 5′-TGATGATTGTTGTCAGCCG-3′, reverse: 5′-TACCTCACCG-3′, Probe: 5′-TACCTGGTGATGCGAGAAAC-3′).
FIGURE 1. IFN-γ significantly upregulates NK2R mRNA level of DCs in a STAT1-dependent manner. (A) Bone marrow-derived DCs were generated from wild-type mice. Quantitative PCR analysis was used to evaluate NK2R mRNA expression level in the DCs treated with IFN-γ (10 ng/ml) for 6 h. Mean and SD were calculated from data of three independent experiments (n = 6/group). (B) NK2R mRNA expression levels in wild-type and Stat1−/− DCs treated with IFN-γ (10 ng/ml) for 6 h were evaluated by PCR. One representative of at least three independent experiments is shown. Scale bars, 5 μm. (C) NK2R protein expression of untreated DCs (left panel; control) or DCs treated with 10 ng/ml IFN-γ for 24 h (right panel) was evaluated by confocal microscopy. One representative of at least three independent experiments is shown. Scale bars, 5 μm. (D) Surface NK2R expression levels of the control and IFN-γ-treated DCs were analyzed by flow cytometry. One representative of at least three independent experiments is shown. (E) Mean fluorescence intensity (MFI; left panel) and percentage (right panel) of NK2R-expressing cells in the control and IFN-γ-treated DCs. Mean and SD were calculated from data of three independent experiments (n = 6/group). *p < 0.05, Student t test.
presence of NK2R antagonist (GR: 10 μM) and OT-II peptide (0, 0.25, 1 μg/ml), OT-I peptide (0, 0.25, 1 μg/ml) for 24–72 h, or OVA protein (0, 25, 100 μg/ml) for 72 h, respectively. IL-2, IFN-γ, IL-4, and IL-17 production was evaluated with the Immunoassay kits.

ELISA
IL-2, IFN-γ, IL-4, IL-17, TNF-α, and IL-6 levels in the culture supernatants were measured using OptEIA mouse IL-2, IFN-γ, IL-4, IL-17, TNF-α, and IL-6 ELISA kits, respectively (BD Biosciences). IFN-α and IFN-β levels in the culture supernatants were measured with VeriKine Mouse IFN-α and IFN-β ELISA kits (PBL InterferonSource, Piscataway, NJ). ELISA kits were used according to the manufacturer’s instructions. The NKA level was determined using a Neurokinin A Enzyme Immunoassay kit (Peninsula Laboratories, San Carlos, CA).

Statistical analysis
All experiments were independently repeated at least three times. Mean and SD were calculated for in vitro murine data, and mean and SE were calculated for in vivo murine and in vitro human data. Significant differences in the results were determined by the two-tailed Student t test; p < 0.05 was considered statistically significant.

Results
IFN-γ stimulation enhances NK2R expression level of DCs
To address the effect of neuropeptide signaling on DC-mediated immune responses, we first investigated the expression level of a receptor for NKA, NK2R, on DCs in a type 1 immune condition. DCs generated from bone marrow cells of wild-type mice were stimulated with recombinant murine IFN-γ in vitro. As a result, IFN-γ treatment significantly induced NK2R mRNA expression on DCs (Fig. 1A, 1B). The NK2R mRNA induction by IFN-γ stimulation was remarkably reduced in STAT-1–deficient DCs (Fig. 1B). These data indicate that IFN-γ stimulation induces NK2R mRNA expression of DCs in a STAT-1–dependent manner. In addition, we found that LPS stimulation induced NK2R mRNA expression on DCs (Supplemental Fig. 1A). The LPS-dependent augmentation of NK2R mRNA level was not observed in IFNAR1- or STAT-1–deficient DCs (Supplemental Fig. 1A, 1B). These data suggest that type 1 IFN produces after LPS stimulation induced NK2R mRNA expression of DCs by an autocrine mechanism in a STAT-1–dependent manner.

We further evaluated whether IFN-γ enhanced NK2R protein expression levels of DCs. Confocal microscopy showed that NK2R protein level was enhanced in the IFN-γ–stimulated DCs (Fig. 1C). We confirmed that both the surface expression levels of NK2R on DCs (Fig. 1D) and the percentage of NK2R-expressing cells were significantly enhanced by IFN-γ stimulation (Fig. 1E). These data suggest that IFN-γ, type 1 cytokine stimulation activates the NK2R-mediated signaling cascade of DCs in a STAT-1–dependent manner.

DCs elevate the NKA, a neuropeptide, level in response to IFN-γ stimulation in vivo and in vitro
In the current study, we found that intranasal injection of IFN-γ into wild-type mice significantly enhanced the NK2R mRNA expression level of lung tissue and the NKA level in BALF, resulting in increased airway inflammation (11). In the current study, we investigated whether DCs were required for the increase in NKA. CD11c-DTR Tg mice were intranasally injected with PBS as a control or DT and then IFN-γ was injected into the mice. We confirmed that CD11c+ populations in the lung tissue and BALF were remarkably reduced by DT treatment (Fig. 2A). At 24 h after the IFN-γ injection, NKA level in BALF was significantly reduced by the depletion of the CD11c+ population (Fig. 2B). These findings suggest that CD11c+ populations, such as pulmonary DCs and alveolar macrophages, contribute to IFN-γ–dependent NKA elevation in the lung. Furthermore, we determined that NKA level in the culture supernatant of bone marrow-derived DCs treated with IFN-γ. As a result, the NKA level increased significantly at 24 h after the IFN-γ stimulation (Fig. 2C). Taken together, these findings indicate that DCs are at least one of the responsible populations for the increase in NKA levels in response to IFN-γ stimulation.

NKA stimulation significantly induces IFN-α/β mRNA expression levels on DCs
We further investigated the effect of NKA on the production of cytokines by DCs. NKA stimulation significantly enhanced mRNA expression levels of both IFN-α (Fig. 3A) and IFN-β (Fig. 3B), whereas IL-12p40 increased only slightly (Fig. 3C), and TNF-α (Fig. 3D) and IL-6 (Fig. 3E) were unchanged. In this study, we confirmed that LPS, a TLR4 ligand, stimulation significantly enhanced IFN-β, IL-12p40, TNF-α, and IL-6 but not IFN-α. These findings suggest that NKA stimulation induces type 1 IFN mRNAs by DCs. Furthermore, we evaluated IFN-α, IFN-β, IL-12p70, TNF-α, and IL-6 protein production by NKA-stimulated DCs. However, we could not detect these protein production levels after the NKA stimulation in the present experiments (data not shown).

Transduction of NK2R gene into DCs enhances Ag-specific IL-2 production by CD4+ T cells
To evaluate the effects of NK2R-mediated NKA stimulation on DC functions, we constructed the pMX-FLAG-NK2R-IRES-GFP ret-
roviral vector. We then confirmed the surface expression level of FLAG-tagged NK2R protein in Plat E cells by flow cytometry (data not shown) and alteration of the morphology of NK2R-overexpressed RAW264.7 cells compared with Mock-RAW264.7 cells after NKA stimulation (Supplemental Fig. 2A).

We transduced NK2R gene into DCs with a retrovirus-infection system, and the Mock- or NK2R-transduced DCs were isolated using a cell sorter. We found that the NK2R-DCs enhanced surface MHC class II expression level after NKA stimulation compared with Mock-DCs (Fig. 4A), although IL-12 production by Mock- or NK2R-DCs was not observed by flow cytometry (Supplemental Fig. 2C). Then, we cocultured OT-II CD4+ T cells with Mock- or NK2R-transduced DCs in the presence or absence of OT-II peptides plus NKA or vehicle. The transduction of NK2R gene into DCs appeared to promote the Ag-specific responses to CD4+ T cells (Fig. 4B). Then, we evaluated the proliferation by [3H]thymidine-incorporation assay and confirmed that NK2R-DCs significantly enhanced cell proliferation compared with Mock-DCs. In addition, NKA stimulation increased the enhancement of cell proliferation (Fig. 4C). Furthermore, we examined IL-2 production by CD4+ T cells cocultured with Mock- or NK2R-transduced DCs in the presence of NKA with or without OT-II peptide. NK2R-DCs significantly enhanced Ag-specific IL-2 production by CD4+ T cells compared with Mock-DCs. NKA stimulation augmented IL-2 production by CD4+ T cells cocultured with NK2R-DCs (Fig. 4D). These data suggest that NK2R-dependent NKA stimulation increases Ag-presentation of DCs to activate CD4+ T cells.

In the present experiments, the proliferation and IL-2 production by CD4+ T cells were significantly increased by the NK2R transduction into DCs, even in the absence of extraneous NKA stimulation. We found that the culture medium with 10% FCS contained NKA, as shown in the normal culture condition (Fig. 2C), suggesting that the NKA in the culture medium also affected MHC class II expression of NK2R-transduced DCs without the further addition of NKA.

**Blockade of NK2R-mediated signaling pathway significantly inhibits Ag-specific CD4+ and CD8+ T cell responses**

To confirm the effect of NKA–NK2R signaling on Ag-specific T cell responses, we cultured spleen cells obtained from OT-II or OT-I mice in the presence of NK2R antagonist GR for 48 h. Immune responses to each OVA Ag peptide were evaluated by [3H]thymidine-incorporation assay. The Ag-specific proliferation of spleen cells from OT-II and OT-I mice was significantly suppressed by the addition of GR (10 μM; Supplemental Fig. 3A).

We confirmed that there was no difference in the ratio of 7AAD+ Annexin V+ apoptotic cells between GR and DMSO control groups at 8 h (Supplemental Fig. 3B).

Then, we cocultured OT-II CD4+ T cells with DCs in the presence of GR (10 μM). The blockade of NK2R-mediated signaling by antagonist remarkably reduces IFN-γ and IL-2 production by CD4+ T cells in the presence of OT-II peptide or OVA protein (Fig. 5A). Under the same conditions, neither IL-17 nor IL-4 production was observed in the present coculture system (data not shown). Furthermore, we examined Ag-specific responses of OT-I CD8+ T cells cocultured with DCs in the presence of GR. The blockade of NK2R-mediated signaling also suppresses IFN-γ production by CD8+ T cells after stimulation with OT-I peptide or OVA protein (Fig. 5B).
Thus, these findings strongly suggest that the NKA–NK2R-signaling cascade is involved in DC-mediated type 1 immunity including Ag-specific CD4+ and CD8+ T cell responses. Human DCs induce NK2R and TAC1 genes after IFN-γ stimulation

Finally, we examined whether human DCs induce expression of NK2R (hNK2R) and TAC1 (hTAC1) after IFN-γ stimulation. CD11c+, HLA class II-, and HLA class I-expressing DCs were generated from adherent cells of human PBMCs and stimulated with recombinant human IFN-γ for 24 h. As a result, we confirmed that both hNK2R protein level (Fig. 6A) and surface expression levels (Fig. 6B, 6C) were remarkably enhanced by IFN-γ stimulation, similar to the case observed in mouse DCs. In this study, we found that both hNK2R and hTAC1 mRNA expression levels were significantly enhanced by IFN-γ stimulation (Fig. 6D, 6E). Furthermore, human DCs enhanced HLA class II expression levels after NKA plus IFN-γ stimulation compared with IFN-γ alone, whereas NKA stimulation did not induce cytokine production (data not shown). These findings suggest that NKA–NK2R signaling could regulate DC function in human immunity.

Discussion

Excitatory transmitters, such as tachykinins including SP and NKA, are widely distributed within both the central and the peripheral nervous systems. Although the tachykinins were initially considered neurotransmitters, many articles reported that expression of their receptors, NK1R and NK2R, was observed in non-neural tissues, including endothelial cells, fibroblasts, smooth
muscle cells, and inflammatory cells, suggesting that tachykinins function between the nervous system and other organs (27–33).

In this study, we found that both murine and human DCs, one of the most powerful APCs, significantly enhanced NKA production levels and expressed NK2R after IFN-γ stimulation (Figs. 1, 6). In addition, stimulation of DCs with TLR ligands, such as LPS and poly I:C, which mimic bacterial and virus infection, respectively, enhanced NK2R expression levels (Fig. 6, Supplemental Fig. 1).

In addition, we revealed in this study that murine DCs stimulated with NKA significantly induced mRNA expression of IFN-α and IFN-β, type 1 IFNs (Fig. 3). Although the protein expressions of these cytokines were not detected in the present experiments, we confirmed the blocking effects of anti-IFN-β and anti-IL-12 mAbs on NKA-dependent augmentation of IFN-γ production after OVA Ag stimulation (data not shown). Therefore, we speculated that NKA-mediated cytokine production might function in the microenvironment, including cell-to-cell interaction. Taken together, these findings strongly suggest that NK2R-mediated neuropeptide signaling is involved in host immune responses, including cytokine production and Ag presentation by DCs.

Previous works indicated that blockade of NK1R influences host responses against various infections. In salmonellosis, mice treated with an SP antagonist have increased bacterial burden and reduced IFN-γ and IL-12 production compared with appropriate controls (38, 39). Mice lacking NK1R reduced schistosomiasis granuloma formation (40). In a posttreatment-reactive encephalopathy model of Trypanosoma brucei, NK1R-deficient mice have a significantly reduced clinical impairment, but a more severe neuroinflammatory response, compared with wild-type mice (41). The critical roles of SP were reported in T cell-mediated experimental autoimmune encephalomyelitis models (42, 43). The findings strongly suggest that the SP–NK1R–signaling cascade contributes to type 1 immunity, as well as inflammatory responses. Although NK1R is ubiquitously expressed in addition to the CNS, it was demonstrated that NK2R is mostly expressed in peripheral tissues (29–33). Although the precise function of the NKA–NK2R–signaling pathway, especially in immune responses, is less-well known compared with that of NK1R, we demonstrated in this study that NK2R-mediated NKA stimulation enhances DC function, such as IFN-α/β production (Fig. 3A, 3B), MHC class II expression (Fig. 4A), and Ag presentation to CD4+ and CD8+ T cells (Figs. 4B, 4C, 5).

NK2R, as well as NK1R, belongs to the rhodopsin-like family I of G protein-coupled receptors, which plays a crucial role in intracellular signaling (27, 28). Previously, we indicated that the TRIF–GEFH1–RhoB pathway regulated MHC class II expression on DCs, which was required for the subsequent CD4+ T cell activation (44). In this study, we indicated that the surface MHC...
class II expression level of NK2R-transduced DCs was increased by NKA stimulation (Fig. 4A). Moreover, the morphology of NK2R-transduced DCs was altered by the addition of NKA (Supplemental Fig. 2B) in addition to NK2R-transduced RAW264.7 cells (Supplemental Fig. 2A). These findings suggest that NK2R-mediated signaling in DCs might be related to membrane trafficking of MHC class II vesicles and alteration of cytoskeleton mediated by a posttranscriptional mechanism.

NKA stimulation significantly enhanced mRNA expression levels of type I IFNs of DCs rather than IL-12p40, although it did not induce mRNA expression levels of TNF-α and IL-6 (Fig. 3, Supplemental Fig. 2C). In general, plasmacytoid DCs induce both IFN-α and IFN-β production in response to foreign nucleic acids through TLR7 and TLR9 (45). In contrast, conventional DCs and macrophages produce IFN-β and various proinflammatory cytokines by TLR3, TLR7, and TLR9 ligands after stimulation (46). Although DCs generated from bone marrow with GM-CSF in this study showed phenotypes of conventional DCs, IFN-α and IFN-β mRNA expression was induced by NKA stimulation (Fig. 3, 3B). The present data suggest that NK2R-dependent neuropeptide signaling uses a different mechanism than the TLR-dependent pathway for cytokine production by conventional DCs.

Recently, we demonstrated that intranasal administration of IFN-γ remarkably induced NKA and NK2R expression in the lung tissues and blockade of NKA–NK2R signaling significantly suppressed IFN-γ–induced AHR and the symptom of Th1 cell-transferred asthma model (11). These findings suggested that the NKA–NK2R cascade must be related to neuro-immune cross-talk in type I immunity including airway inflammation. We found in this study that depletion of CD11c+ cells from lung tissues remarkably reduced the production level of NKA after intranasal IFN-γ injection in vivo (Fig. 2B). We also confirmed that NK2R expression was induced in alveolar macrophages collected from BALF, in addition to macrophages from peritoneal exudate cells and spleen CD11c+ DCs after IFN-γ stimulation (data not shown). Thus, we speculated that both pulmonary DCs and alveolar macrophages might regulate type I immunity through the NK2R-mediated signaling pathway in the lung. Then, we focused on the precise effect of NKA–NK2R signaling on the immunological function of DCs. NK2R-transduced DCs increased MHC class II levels, resulting in the enhanced proliferation of CD4+ T cells and IL-2 production by CD4+ T cells after the Ag stimulation (Fig. 4). In addition, NK2R selective antagonist strongly suppressed both IFN-γ and IL-2 production by CD4+ T cells and IFN-γ production by CD8+ T cells cocultured with Ag-loaded DCs (Fig. 5). IL-4 and IL-17 production by CD4+ T cells did not increase in these experiments (data not shown). Thus, NK2R-dependent neuropeptide signaling would be involved in host inflammatory responses induced by type I immunity via the augmentation of DC functions (Supplemental Fig. 4).

Chronic inflammatory pulmonary disorders, such as various infections, and severe asthma, including childhood asthma and COPD of smokers, have become a major public health problem worldwide. Although the pathology of severe asthma occasionally shows steroid drug resistance, the precise mechanisms remain unknown compared with Th2-mediated bronchial asthma. We established Th1-, Th2-, and Th17-dependent asthma models by adoptive transfer of these effector cells into wild-type mice (9, 10) and confirmed that the Th1 and Th17 models were steroid resistant, whereas the Th2-mediated asthma model was steroid sensitive. Then, we confirmed that administration of NK2R antagonist significantly blocked the Th1 cell-dependent AHR elevation. Therefore, we have a high expectation that blockade of the NKA–NK2R–signaling pathway would be a powerful therapy for patients with steroid-resistance disorders, including severe inflammatory diseases.

In the present work, we found that human DCs also induced expression of hNKA and hTAC1 mRNAs in response to IFN-γ (Fig. 6). Furthermore, stimulation of the TLR3 ligand poly I:C enhanced both genes. Although human CD11c+ and HLA class II+ cells were possible contaminants of both monocyte and macrophages in the present experiments, these findings indicate that NK2R-dependent cross-talk between the neural and immune systems would function in human subjects. Therefore, we are now evaluating the effect of NK2R antagonist on the activation of human immune cells and confirming the several suppression effects of cytokine production by human DCs and T cells.

We conclude that NK2R-dependent neuropeptide signaling activates DC-mediated immune responses, suggesting that the NKA/NK2R-mediated signaling pathway would be a promising target in therapy for patients with chronic inflammation caused by excessive activation of type I immunity, including steroid-resistant severe asthma.

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Disclosures

The authors have no financial conflicts of interest.

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