Autocrine hemokinin-1 functions as an endogenous adjuvant for IgE-mediated mast cell inflammatory responses

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Background: Efficient development of atopic diseases requires interactions between allergen and adjuvant to initiate and amplify the underlying inflammatory responses. Substance P (SP) and hemokinin-1 (HK-1) are neuropeptides that signal through the neurokinin-1 receptor (NK1R) to promote inflammation. Mast cells initiate the symptoms and tissue effects of atopic disorders, secreting TNF and IL-6 after FcεRI cross-linking by antigen-IgE complexes (FcεRI-activated mast cells [FcεRI-MCs]). Additionally, MCs express the NK1R, suggesting an adjuvant role for NK1R agonists in FcεRI-MC–mediated pathologies; however, in-depth research addressing this relevant aspect of MC biology is lacking.

Objective: We sought to investigate the effect of NK1R signaling and the individual roles of SP and HK-1 as potential adjuvants for FcεRI-MC–mediated allergic disorders.

Methods: Bone marrow–derived mast cells (BMMCs) from C57BL/6 wild-type (WT) or NK1R$^{-/-}$ mice were used to investigate the effects of NK1R signaling on FcεRI-MCs. BMMCs generated from *Tac1*$^{-/-}$ mice or after culture with *Tac4* small interfering RNA were used to address the adjuvancy of SP and HK-1. WT, NK1R$^{-/-}$, and c-Kit$^{W\text{-}sh/W\text{-}sh}$ mice reconstituted with WT or NK1R$^{-/-}$ BMMCs were used to evaluate NK1R signaling on FcεRI-MC–mediated passive local and systemic anaphylaxis and on airway inflammation.

Results: FcεRI-activated MCs upregulated NK1R and HK-1 transcripts and protein synthesis, without modifying SP expression. In a positive signaling loop HK-1 promoted TNF and IL-6 secretion by MC degranulation and protein synthesis, the latter through the phosphoinositide 3-kinase/Akt/nuclear factor κB pathways. *In vivo* NK1R signaling was necessary for the development of passive local and systemic anaphylaxis and airway inflammation.

Conclusions: FcεRI stimulation of MCs promotes autocrine secretion of HK-1, which signals through NK1R to provide adjuvancy for efficient development of FcεRI-MC–mediated disorders. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Hemokinin-1, substance P, neurokinin-1 receptor, mast cells, IgE, FcεRI, TNF, IL-6, passive anaphylaxis, airway inflammation*

The immune system is designed to eliminate foreign antigens while maintaining tissue integrity. Innate and adaptive immune responses should effectively be resolved after the neutralization of foreign antigen to accomplish these functions. Adjuvant-mediated increases in the intensity or prolongation of inflammatory reactions result in fatal outcomes in the case of anaphylaxis and irreversible tissue damage with function loss. Inflammatory responses are amplified by neuropeptides from the tachykinin family, such as substance P (SP) and hemokinin-1 (HK-1).[1] Both neuropeptides exert their effects by signaling through the neurokinin-1 receptor (NK1R), a 7-transmembrane domain G protein–coupled receptor (GPCR).[2] NK1R signaling mediates pain, inflammation, and immune function, the latter through dendritic cell and monocyte activation,[3-6] chemotaxis and cytotoxicity of natural killer cells,[7,8] and differentiation and survival of pro-T and pro-B lymphocytes.[9-12] Previous reports on SP and HK-1 proinflammatory function and the observation that SP expression is increased in autoimmune disorders[13-15] highly suggest an adjuvant role for NK1R agonists.

Peripheral tissue-resident mast cells (MCs) express the NK1R[16] and are ideally positioned to respond to foreign antigens with innate and adaptive immune functions.[17,18] Activation of FcεRI-activated mast cells (FcεRI-MCs) is central to their pathologic inflammatory function. Cross-linking of surface FcεRI in MCs initiates a biphasic inflammatory response comprising immediate degranulation with release of stored proinflammatory mediators and delayed secretion of *de novo*–synthesized proinflammatory cytokines. FcεRI-MCs release TNF and IL-6, which trigger anaphylaxis and mediate the symptoms and tissue effects of chronic atopic disorders.[17,18]

Mechanistically, FcεRI activation recruits Src family kinases[18] to activate phosphoinositide 3-kinase (PI3K) and phospholipase C cascades that interconnect with intracellular signaling pathways initiated by GPCR.[2,18] Accordingly, interactions between FcεRI and NK1R signaling might regulate MC inflammatory functions. Although scarce reports have associated SP with IgE-independent MC functions,[19-21] the mechanisms and individual roles of NK1R agonists in the biology and function of FcεRI-MCs remain unknown. Furthermore, to our knowledge, information regarding the contribution of HK-1 to MC inflammatory functions is lacking.

In the present work we demonstrate that signaling murine MCs through FcεRI upregulates (1) expression of the NK1R, (2) transcription of the HK-1 gene *(Tac4)*, and (3) synthesis of HK-1, without modifying transcription of the SP gene *(Tac1)* or secretion of SP peptide. In an autocrine/paracrine positive signaling loop, binding the NK1R by HK-1 is critical for FcεRI-mediated

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Abbreviations used
BAL: Bronchoalveolar lavage
BMMC: Bone marrow–derived mast cell
DNP-HSA: Dinitrophenyl–human serum albumin
EPO: Eosinophil peroxidase
ERK: Extracellular signal-regulated kinase
FcεRI-MC: FcεRI-activated mast cell
FcεR1-BMMC: FcεRI-activated bone marrow mast cell
GPCR: G protein–coupled receptor
HK-1: Hemokinin-1
JNK: c-Jun N-terminal kinase
MC: Mast cell
MPO: Myeloperoxidase
NF-κB: Nuclear factor κB
NK1R: Neurokinin-1 receptor
OVA: Ovalbumin
PCA: Passive cutaneous anaphylaxis
PFA: Paraformaldehyde
PSA: Passive systemic anaphylaxis
PI3K: Phosphoinositide 3-kinase
PMN: Polymorphonuclear
SP: Substance P
WT: Wild-type

MC secretion of granule stored *de novo* synthesized TNF, leading to *in vivo* initiation of local and systemic anaphylaxis, as well as development or maintenance of airway inflammation.

METHODS

Supplemental information can be found in the Methods section in this article's Online Repository at www.jacionline.org.

Mice

Female wild-type (WT) C57BL/6 and B6.Cg Tac1$^{tm1Bm/j}$ (*Tac1*$^{-/-}$) mice (8-12 weeks old) were purchased from the Jackson Laboratory (Bar Harbor, Me) and rested for 1 week before use. C57BL/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mice were initially purchased from Jackson Laboratories and bred in the University of Pittsburgh's Animal Facility. NK1R$^{-/-}$ mice, initially provided by Dr Christopher Paige, University of Toronto, have been backcrossed to homozygosity by breeding 8 generations before use. Studies were performed according to Institutional Animal Care and Use Committee approval of protocols and procedures (University of Pittsburgh).

Statistical analysis

Data were analyzed by using 1- or 2-way ANOVA with Bonferroni *post hoc* analysis with GraphPad Prism version 5.0 software (GraphPad Software, La Jolla, Calif). When only 2 groups were compared, significant differences were determined by using the 2-tailed Student *t* test. A *P* value of less than .05 was considered significant.

RESULTS

NK1R signaling enhances FcεRI-initiated MC functions

We analyzed the presence of the functional NK1R through detection of its intracellular C-terminus motif[2] in IL-3–derived WT C57BL/6 bone marrow–derived mast cells (BMMCs). The basal level of *NK1R* expression was low in nonactivated BMMCs, as reported previously.[16] However, FcεRI activation of BMMCs significantly increased NK1R mRNA and protein expression (Fig 1, *A* and *B*). It has been shown that differentiation of BMMCs with high concentrations of IL-4 increases NK1R expression.[16] Because FcεRI activation causes release of IL-4 from BMMCs (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org.),[17] we hypothesized that autocrine IL-4 might play a role in the regulation of NK1R expression. Inhibition of autocrine IL-4 with neutralizing anti–IL-4 antibody inhibited FcεRI-driven NK1R expression (see Fig E1, *B*). In contrast, BMMCs cultured with exogenous IL-4 without FcεRI activation were unable to further increase NK1R expression (see Fig E1, *C*). Together, these results indicate that in our working conditions, NK1R expression in BMMCs is regulated by autocrine IL-4 secretion initiated by FcεRI signaling.

Then we investigated IL-4–independent signaling pathways regulating NK1R expression in FcεRI-BMMCs, including nuclear factor κB (NF-κB), c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase (ERK), which regulate NK1R expression in dendritic cells[3,4] and other cell types.[22,23] Inhibition of NF-κB and JNK, but not ERK, reduced *NK1R* mRNA induced by FcεRI ligation (Fig 1, *B*), demonstrating that FcεRI ligation of MCs promotes NK1R expression through common intracellular pathways described in other cell types.[3,4,22,23]

Ca^{2+}-dependent FcεRI-MC degranulation is potentiated by certain GPCR agonists.[17,18] Because the NK1R is a GPCR, we analyzed whether it was involved in Ca^{2+} flux and MC degranulation by comparing these 2 functions in FcεRI-BMMCs generated from WT or NK1R$^{-/-}$ mice. Both BMMC strains had comparable differentiation and maturation stages according to expression of c-Kit and FcεRI (see Fig E2 in this article's Online Repository at www.jacionline.org.), and they displayed similar changes in intracellular Ca^{2+} levels (Fig 1, *C*). However, fewer NK1R$^{-/-}$ BMMCs degranulated compared with WT BMMCs (Fig 1, *D*), irrespective of antigen concentration (Fig 1, *E*). Conversely, degranulation of MCs induced with compound 48/80, an independent GPCR signaling stimulus,[24] triggered robust degranulation in both WT and NK1R$^{-/-}$ BMMCs. These data demonstrate that the NK1R belongs to the group of GPCR family members that potentiate FcεRI-mediated MC degranulation.

In line with deficient degranulation, NK1R$^{-/-}$ FcεRI-BMMCs had reduced TNF and IL-6 secretion compared with WT FcεRI-BMMCs (Fig 1, *F*). This effect was receptor specific because it was prevented by pretreatment with the NK1R antagonists RP 67,580 (see Fig E3, *A* and *B*, in this article's Online Repository at www.jacionline.org.) and L733,060 (see Fig E3, *C* and *D*). Collectively, these data demonstrate that NK1R stimulation represents a downstream component of the proinflammatory signaling cascade initiated by FcεRI activation of MCs.

MCs secrete HK-1

The previous results, obtained with a highly pure MC population and without addition of exogenous NK1R agonists, suggested that MCs are the source of NK1R agonists, which increase their inflammatory functions in a highly regulated fashion. Therefore we investigated the capacity of FcεRI-BMMCs to synthesize and secrete HK-1 and SP. We analyzed the regulation of *Tac4* and *Tac1* transcripts (encoding HK-1 and SP, respectively) and secretion of HK-1 and SP peptides in WT untreated BMMCs (control) and FcεRI-BMMCs. *Tac4* and *Tac1* mRNAs were detected in untreated BMMCs, but only *Tac4* transcripts increased in FcεRI-BMMCs (Fig 2, *A*). Accordingly, *Tac4*