# Cannabinoid receptor 1 controls human mucosal-type mast cell degranulation and maturation *in situ*

**Koji Sugawara, MD, PhD,[a,b] Nóra Zákány, MS,[a,c]* Torsten Hundt, DiplPharm,[a]* Vladimir Emelianov, MD, PhD,[a] Daisuke Tsuruta, MD, PhD,[b] Christian Schäfer, MD,[d] Jennifer E. Kloepper, DVM,[a] Tamás Bíró, MD, PhD,[c] and Ralf Paus, MD[a,e]** *Lübeck, Germany, Osaka, Japan, Debrecen, Hungary, and Manchester, United Kingdom*

**Background: Because many chronic inflammatory and allergic disorders are intimately linked to excessive mast cell (MC) numbers and activation, it is clinically important to understand the physiologic mechanisms preventing excess MC accumulation/degranulation in normal human tissues.**
**Objective: Because endocannabinoids are increasingly recognized as neuroendocrine regulators of MC biology, we investigated how cannabinoid receptor (CB) 1 signaling affects human mucosal-type mast cells (hMMCs).**
**Methods: Using organ-cultured nasal polyps as a surrogate tissue for human bronchial mucosa, we investigated how CB1 stimulation, inhibition, or knockdown affects hMMC biology using quantitative (immuno)histomorphometry and electron microscopy.**
**Results: Kit$^+$ hMMCs express functional CB1 *in situ*. Blockade of CB1 signaling (with the specific CB1 antagonist N-(piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-(4-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide [AM251] or CB1 gene knockdown) enhanced hMMC degranulation and increased total numbers without affecting their proliferation *in situ*. This suggests that inhibiting CB1 signaling induces hMMC maturation from resident progenitor cells within human mucosal stroma. hMMC maturation was induced at least in part through upregulating stem cell factor production. Both the prototypic endocannabinoid anandamide and the CB1-selective agonist arachidonyl-2-chloroethylamide effectively counteracted secretagogue-triggered excessive hMMC degranulation.**
**Conclusions: The current serum-free nasal polyp organ culture model allows physiologically and clinically relevant insights into the biology and pharmacologic responses of primary hMMCs *in situ*. In human airway mucosa hMMC activation and maturation are subject to a potent inhibitory endocannabinoid tone through CB1 stimulation. This invites one to target the endocannabinoid system in human airway mucosa as a novel strategy in the future management of allergic diseases.**
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***Key words:*** *Nasal polyp, mucosa, organ culture, endocannabinoid, cannabinoid receptor, mast cell, stem cell factor, c-Kit, tryptase, chymase*

Although the central role of human mucosal-type mast cells (hMMCs) in patients with respiratory diseases, including allergic rhinitis and asthma, is well appreciated,[1,2] most research on hMMCs relies on isolated cells obtained during bronchial lavage or cell lines.[3,4] Studies that examine and manipulate primary hMMCs within their natural tissue habitat, the respiratory tract mucosa, are scarce.[5-7] However, it is crucial to study hMMCs *in situ* rather than in isolated cell cultures because mast cell (MC) functions (including activation, degranulation, maturation, proliferation, and apoptosis) are critically influenced by their immediate tissue environment and MCs greatly affect the tissue they reside in, namely the airways.[8-12] For such *in situ* studies of hMMCs, nasal polyps (NPs) have long offered a very attractive yet still regrettably underappreciated assay option for clinically relevant *in situ* MC research in the human system.[5,6]

NPs represent polypoidal masses that arise mainly from nasal and paranasal mucous membranes and are frequently associated with allergic rhinitis and other "atopic" diseases.[13,14] Because the nasal mucosa forms one functional continuum with the upper respiratory tract mucosa,[15,16] organ culture of NPs can serve as an easily accessible, well-defined, and abundantly available surrogate tissue for the much less readily obtainable bronchial mucosa and its hMMC populations.[5,6] Moreover, hMMCs also play an important role in the pathogenesis of NP formation, as such,[2,5,17-19] so that MC research in organ-cultured NPs simultaneously allows one to investigate one of the most common clinical problems of upper respiratory tract care.

Previous human NP organ culture models[5,6,20] are limited by relatively rapid NP decay, the presence of bovine serum in the culture medium, or both; require special matrix support systems; and/or have not systematically addressed key MC biology questions, such as the physiologic controls of hMMC maturation and activation *in situ*. Therefore we aimed to develop a very simple, serum-free organ culture system that (1) prolongs NP tissue viability *in vitro*, (2) permits the quantitative study of key MC research parameters *in situ*, and (3) permits instructive functional and mechanistic studies, including pharmacologic manipulation and gene knockdown, of primary hMMCs *in situ*.

As a potentially interesting system that might control hMMC functions, we turned to the endocannabinoid system (ECS). The ECS is composed of cannabinoid receptors (CBs), their endogenous ligands, and enzymes responsible for endocannabinoid

From [a]the Department of Dermatology, University of Lübeck; [b]the Department of Dermatology, Osaka City University Graduate School of Medicine, Osaka; [c]the Department of Physiology, DE-MTA "Lendület" Cellular Physiology Research Group, MHSC, RCMM, University of Debrecen; [d]E.N.T. Clinic Lübeck, Schuesselbuden, Lübeck; and [e]the Dermatology Centre, Salford Royal NHS Foundation Trust, Institute of Inflammation and Repair, University of Manchester.
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Corresponding author: Koji Sugawara, MD, PhD, Department of Dermatology, Osaka City University Graduate School of Medicine, 1-4-3, Asahimachi, Abeno-ku, Osaka, 545-8585, Japan. E-mail: kojilaminin@yahoo.co.jp.
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*Abbreviations used*

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| ACEA: | Arachidonyl-2-chloroethylamide |
| AEA: | N-arachidonoylethanolamide (anandamide) |
| AM251: | N-(piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-(4-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide |
| CB: | Cannabinoid receptor |
| CRH: | Corticotropin-releasing hormone |
| ECS: | Endocannabinoid system |
| hMMC: | Human mucosal-type mast cell |
| LDH: | Lactate dehydrogenase |
| MC: | Mast cell |
| NP: | Nasal polyp |
| SCF: | Stem cell factor |
| TUNEL: | Terminal dUTP nick-end labeling |

synthesis and degradation.[21-25] Components of the ECS are increasingly recognized as important neuroendocrine regulators of MC biology.[21,26-28] Notably, we have recently reported in this journal that the ECS limits excessive human skin MC activation and maturation through CB1-mediated signaling *in situ*.[29] However, the role of CB-mediated signaling in hMMCs still remains largely unknown (see Supplementary Text S1 in this article's Online Repository at www.jacionline.org). Therefore we investigated the effects of CB1 stimulation/blockade on hMMC biology within organ-cultured human nasal mucosa.

## METHODS

### Human NP organ culture

Human NP samples were obtained from 7 male and 3 female subjects (age, 23-80 years; average, 39.5 years) undergoing elective surgery for nasal obstruction (polypectomy). Human tissue collection and handling was performed according to Declaration of Helsinki guidelines, with institutional research ethics committee approval (University of Lübeck) and written informed consent. Freshly isolated NPs were cut into small pieces ($6 \times 6 \times 6 - 10 \times 10 \times 10$ mm) and maintained in supplemented serum-free William E medium.[29-32] NPs were first incubated overnight to adapt to culture conditions, after which the medium was replaced and vehicle or test substances were added. For the organ culture with substance P, compound 48/80, and corticotropin-releasing hormone (CRH), NPs were first treated with N-arachidonoylethanolamide (anandamide [AEA]; 30 μmol/L) or arachidonyl-2-chloroethylamide (ACEA; 30 μmol/L) for 1 day after the overnight incubation. Then the NPs were treated with substance P ($10^{-10}$ mol/L), compound 48/80 (10 μg/mL),[29] or CRH ($10^{-7}$ mol/L)[31] in combination with AEA or ACEA for an additional 1 day. After NP organ culture for the indicated time, tissue was processed for cryosectioning or paraffinized sectioning and histochemistry, immunohistochemistry, or transmission electron microscopy. Data from test and control groups within one set of experiments were generated by using only NPs from the same patient.

### CB1 knockdown

CB1 silencing in organ-cultured human NPs was performed by using a previously reported method.[29]

### Immunohistochemistry/immunofluorescence microscopy

For the detection of CB1, stem cell factor (SCF), Kit, FcεRIα, tryptase, and chymase immunohistochemistry, paraffin-embedded sections were used. For the immunofluorescence study for Kit, CB1, and SCF, cryoembedded sections were also used. For further details, see the Methods section in this article's Online Repository at www.jacionline.org.

hMMCs were defined as degranulated when more than 5 histochemically or immunohistologically detectable MC granules could clearly be observed outside the MC membrane (see representative degranulated MCs in Fig E1, *A*, in this article's Online Repository at www.jacionline.org).[29,31]

### Quantitative immunohistomorphometry and transmission electron microscopy

Quantitative immunohistomorphometry of the observed immunoreactivity patterns in defined reference areas were assessed according to previously described principles[29-32] by using ImageJ software (National Institutes of Health, Bethesda, Md). Transmission electron microscopy was performed as previously reported.[29]

### Statistical analysis

Data were analyzed by using either the Mann-Whitney *U* test for unpaired samples or 1-way ANOVA (Bonferroni multiple comparison test) with Prism 4.0 software (GraphPad Prism; GraphPad Software, San Diego, Calif). *P* values of less than .05 were regarded as significant. All data in the figures are expressed as means ± SEMs.

## RESULTS

### Human NPs can be organ cultured for at least 7 days

First, we adapted the long-term organ culture of human skin and hair follicles[29-32] to the organ culture of human NPs using supplemented, serum-free William E medium. This simple and cost-efficient assay preserved NP architecture for at least 7 days (later time points were not examined; Fig 1, *A* and *B*). Because hematoxylin stains viable cells,[33-35] the large number of hematoxylin-positive cells still visible in all NP compartments even after 7 days of organ culture suggests reasonably good general tissue viability over time. Although the cellularity in the lamina propria, but not in the epithelium, was significantly decreased (Fig 1, *C* and *D*), the NP tissue architecture was reasonably well preserved, and even mucosal cilia in the NP epithelium, as well as blood vessels and collagen fibers of the NP stroma (lamina propria), were partially conserved (Fig 1, *A* and *B*).

After a temporary decrease subsequent to the trauma of tissue dissection, at day 7, cell proliferation in the NP epithelium quickly recovered and reached initial day 0 levels (Fig 1, *E*). In contrast, cell proliferation within the NP lamina propria remained stable throughout the examined organ culture period (Fig 1, *F*). Although intermittently increased stromal cell apoptosis was observed at day 3 (Fig 1, *G*), general tissue decay parameters, such as percentage of apoptotic (terminal dUTP nick-end labeling [TUNEL]–positive) cells and lactate dehydrogenase (LDH) release, also had stabilized by day 7 (Fig 1, *G* and *H*, and see Fig E1, *B*). However, as previously reported,[5] with increasing organ culture time, the NP stroma became slowly more edematous (Fig 1, *A*). In addition to the reduction of cellularity in the lamina propria at day 7, this needs to be accounted for when calculating cell numbers per reference area in NP organ culture.

### hMMCs are detectable histochemically and immunohistochemically in NP organ culture

Multiple fully granulated (Fig 2, *A*) or degranulated (Fig 2, *B*) hMMCs could be detected histochemically at all examined time points of NP organ culture. For this, Leder esterase (Fig 2, *A*) or Giemsa (Fig 2, *A*) or alkaline Giemsa (see Fig E1, *C*) histochemistry provided optimal cell visualization and morphologic details.

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