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Cholinergic chemosensory cells of the thymic medulla express the bitter receptor Tas2r131

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ABSTRACT

The thymus is the site of T cell maturation which includes positive selection in the cortex and negative selection in the medulla. Acetylcholine is locally produced in the thymus and cholinergic signaling influences the T cell development. We recently described a distinct subset of medullary epithelial cells in the murine thymus which express the acetylcholine-synthesizing enzyme choline acetyltransferase (ChAT) and components of the canonical taste transduction cascade, i.e. transient receptor potential melastatin-like subtype 5 channel (TRPM5), phospholipase $C\beta_2$, and Gα-gustducin. Such a chemical phenotype is characteristic for chemosensory cells of mucosal surfaces which utilize bitter receptors for detection of potentially hazardous compounds and cholinergic signaling to initiate avoidance reflexes. We here demonstrate mRNA expression of bitter receptors Tas2r105, Tas2r108, and Tas2r131 in the murine thymus. Using a Tas2r131-tauGFP reporter mouse we localized the expression of this receptor to cholinergic cells expressing the downstream elements of the taste transduction pathway. These cells are distinct from the medullary thymic epithelial cells which promiscuously express tissue-restricted self-antigens during the process of negative selection, since double-labeling immunofluorescence showed no colocalization of autoimmune regulator (AIRE), the key mediator of negative selection, and TRPM5. These data demonstrate the presence of bitter taste-sensing signaling in cholinergic epithelial cells in the thymic medulla and opens a discussion as to what is the physiological role of this pathway.

1. Introduction

The thymus is the site where T cells undergo maturation through several stages, which can be roughly divided into positive selection that takes place in the cortex, and negative selection which occurs in the medulla. Positive selection ensures survival of those T cells which react with self-MHC on the surface of epithelial cells, whereas negative selection eliminates those T cells that react with self-antigens presented by medullary epithelial and dendritic cells. Autoimmune regulator (AIRE) plays a key role in negative selection by inducing expression of tissue-restricted antigens in medullary thymic epithelial cells (mTECs) [1–3]. AIRE deficiency results in autoimmunity [1].

T cell maturation is influenced by cholinergic signaling [4,5], and acetylcholine is endogenously synthesized in the thymus [6,7]. Until recently, cellular sources of endogenous acetylcholine in the thymus have not been identified *in situ*. Utilizing a transgenic mouse strain expressing enhanced green fluorescent protein (eGFP) driven by the promotor of the acetylcholine-synthesizing enzyme choline acetyltransferase (ChAT), we identified a novel mTEC phenotype, and these cholinergic cells also express components of the canonical taste transduction cascade, i.e. the G-protein α-gustducin, phospholipase $C\beta_2$ ($PLC\beta_2$), and the cation channel transient receptor potential melastatin-like subtype 5 (TRPM5) [2]. Epithelial cells with such a phenotype have previously been described in various mucosal surfaces. In the nose, trachea and urethra, they utilize the taste transduction cascade to monitor the luminal surface for the presence of potentially hazardous compounds, e.g. bacterial products, and, upon stimulation, they initiate local and general protective reflexes via cholinergic signaling to sensory nerve fibers [3–7]. Based on analogy to these mucosal chemosensory cells and the expression profile of the novel cholinergic mTECs, we provisionally proposed the term thymic cholinergic chemosensory cells (CCCs) for this entity [2]. Direct proof of taste receptor expression, however, has not been provided yet.

α-Gustducin, $PLC\beta_2$, and TRPM5 are downstream elements of the bitter, sweet and umami taste transduction cascade [8–11]. Initial recognition occurs via G-protein coupled taste receptors of the Tas1r

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Table 1
Antibodies used in the study.

Target	Immunogen	Host	Clone	Dilution	Catalog no.	Source
PLCβ_2	C-terminus of PLCβ_2 of human origin	Rabbit	Polyclonal	1:800	sc-206	Santa Cruz Biotechnology, Heidelberg, Germany
TRPM5	H2N-ARDREYLESGLPPSDT-COOH	Rabbit	Polyclonal	1:500	Ø	[17]
AIRE	Internal region of AIRE-1 of human origin	Goat	Polyclonal	1:400	sc-17986	Santa Cruz Biotechnology, Heidelberg, Germany
ChAT	Human placental enzyme	Goat	Polyclonal	1:250	AB144P	Chemicon
K5	Synthetic peptide corresponding to C-terminus of human keratin 5	Rabbit	Monoclonal, clone SP27	1:200	SPB-M3270	Spring, Pleasanton, CA, USA
K8	Synthetic peptide corresponding to C-terminus of human keratin 8	Rabbit	Monoclonal, clone SP102	1:50	SPB-M4020	Spring, Pleasanton, CA, USA
K10	C-terminus of the mouse keratin 10	Rabbit	Polyclonal	1:400	PRB-159P	Covance, Münster, Germany
K14	Synthetic peptide corresponding to C-terminus of human keratin 14	Rabbit	Monoclonal, clone SP53	1:400	SPB-M3534	Spring, Pleasanton, CA, USA
K18	Synthetic peptide corresponding to C-terminus of human keratin 18	Rabbit	Monoclonal, clone SP69	1:200	SPB-M3694	Spring, Pleasanton, CA, USA

and Tas2r families. Bitter tasting substances are perceived via a family of Tas2 receptors that includes more than 30 members in rodents [12]. In view of the lack of specific antibodies against most members of the Tas2r family allowing for immunohistochemical localization, appropriate reporter mouse strains are a valuable tool for identification of extragustatory cells expressing taste receptors. This approach revealed expression of Tas2r131 in a subset of mouse colonic goblet cells, but neither in intestinal enteroendocrine nor in chemosensory brush cells [13]. Tas2r131 expression was also observed in testis, epididymis, sperm, respiratory epithelium and thymus [14]. The identity of these thymic Tas2r131 cells has not been revealed yet. We here set out to determine whether these Tas2r131 expressing thymic cells correspond to the proposed thymic CCCs, and performed additional immunolabeling for AIRE to determine whether these newly characterized cells are a subset of mTECs involved in antigen presentation in the process of negative selection, or represent a novel entity. Antibodies against cytokeratins characteristic for cortical TECs (K8, K18), mTECs (K4, K15), and terminally differentiated epithelial cells (K10) served to further classify Tas2r131-expressing thymic cells.

2. Materials and methods

2.1. Tissues and animals

Generation of Tas2r131-tauGFP mice has been described in detail earlier [15]. Mice (n = 4, aged 10–16 weeks) were anesthetized with Narcoren (Merial, Halbergmoos, Germany) and perfused through the ascending aorta with PBS followed by phosphate-buffered ice-cold 4% paraformaldehyde (PFA). Tissues were removed, postfixed for 2 h in the same fixative, washed repeatedly in PBS, immersed overnight in 30% sucrose at 4 °C, and frozen in melting isopentane. For immunostaining against AIRE, thymi from C57BL/6 mice (n = 4, aged 5–10 weeks) were prepared as described previously [16]. For RT-qPCR, C57BL/6 mice (n = 3, aged 5–11 weeks) were killed by cervical dislocation, thymi were collected and processed as described previously [2].

2.2. Immunohistochemistry

Immunohistochemistry was performed as described previously [16]. Briefly, fixed tissues were cut with a cryostat into 4–10 μm-thick sections, non-specific binding sites were saturated by incubation with a blocking solution (10% horse serum, 0.5% Tween, and 0.1% BSA in PBS (0.005 M phosphate buffer, pH = 7.4, with 0.45% NaCl)), sections were covered with primary antibodies overnight, washed in PBS, and stained with secondary antibody for 1 h. In case of AIRE-TRPM5 double-staining, epitopes were exposed by boiling the sections in citrate buffer (10 mM citric acid in water, pH = 6.0) for 10 min, with subsequent washing in PBS, incubation for 10 min in 0.1% Triton X-100 and washing again in PBS. Antibody sources and dilutions are indicated in Table 1.

2.3. RT-qPCR

RNA extraction and cDNA synthesis were performed as described previously [2]. For qPCR, 0.5–2.5 μl of cDNA were mixed with 12.5 μl of SYBR Green Real-Time PCR Supermix (BioRad, Munich, Germany), 0.75 μl of primer mix (forward + reverse, 20 μM each) (MWG Eurofins, Munich, Germany; sequences are specified in Table 2), and 9.25–11.25 μl of water. The qPCR conditions were 5 min at 95 °C, 40 cycles of 20 s at 95 °C, 20 s at 60 °C, and 20 s at 72 °C followed by 91 cycles of 10 s at 50 °C. K5 was used as a control of PCR efficiency, omission of RT was used as a negative control. PCR products were run on 2% agarose gel in TRIS-acetate-EDTA buffer and visualized under UV light.

3. Results

Tas2r131-tauGFP-positive cells were located in the thymic medulla as shown by immunolabeling with cytokeratin antibodies highlighting the cortical (K8 and K18) and medullary epithelial network (K5 and K14) (Fig. 1). Although surrounded by K5- and K14-positive mTECs, Tas2r131-tauGFP-positive cells themselves displayed neither of these immunoreactivities (Fig. 1A and C). Anti-K8 and -K18 antibodies labeled the epithelial network in the cortex and a small subset of less ramified medullary cells (Fig. 1B and D). Tas2r131-tauGFP-positive cells were among these medullary K8/K18-positive cells (Fig. 1B and D). K10-expressing terminally differentiated cells of murine Hassall's corpuscles-like structures represented a population distinct from Tas2r131-tauGFP-positive cells (Fig 1E).

RT-qPCR with whole thymus confirmed expression of Tas2r131 and showed additional expression of Tas2r105 and Tas2r108, two bitter receptors previously characterized in chemosensory epithelial cells in mucosal surfaces [4–6,18] (Fig. 2).

Table 2
Primers used in the study.

Target	Sequence (5′-3′)	Product length	GenBank accession no.
K5	fw: ccaacctccagaacgccatt rev: ctcctccgtagccagaagaga	291 bp	NM_027011.2
Tas2r105	fw: gactggcttccttctcatcg rev: gcaaacaccccaagagaaaa	284 bp	NM_020501
Tas2r108	fw: tggatgcaaacagtctctgg rev: ggtgagggctgaaatcagaa	158 bp	NM_020502
Tas2r131	fw: gcagtatttataactggaatgctgc rev: aggcgctagttcttgtatggt	177 bp	NM_207030.1

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