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Editorial

Recent progress in revealing the biological and medical significance of the non-neuronal cholinergic system

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ABSTRACT

This special issue of International Immunopharmacology is the proceedings of the Fourth International Symposium on Non-neuronal Acetylcholine that was held on August 28–30, 2014 at the Justus Liebig University of Giessen in Germany. It contains original contributions of meeting participants covering the significant progress in understanding of the biological and medical significance of the non-neuronal cholinergic system extending from exciting insights into molecular mechanisms regulating this system via miRNAs over the discovery of novel cholinergic cellular signaling circuitries to clinical implications in cancer, wound healing, immunity and inflammation, cardiovascular, respiratory and other diseases.

The Fourth International Symposium on Non-neuronal Acetylcholine was held on August 28–30, 2014 at the Justus Liebig University of Giessen in Germany. This meeting was held jointly with the 3rd Annual Meeting of the LOEWE Research Consortium *Non-neuronal Cholinergic Systems* that had been established at the universities of Giessen, Marburg and Frankfurt. Approximately 120 scientists from 16 countries over 6 continents participated in this meeting (Fig. 1). The participants discussed the most recent development in elucidation of the physiological and pathophysiological roles of non-neuronal acetylcholine (*ACh*) in regulating stem and cancer cells, immunity and inflammation, functioning of cardiac and respiratory systems and the mucocutaneous epithelial barrier as well as discoveries of novel cholinergic ligands in animals and bacteria. The articles of the symposium participants covering a large variety of biological and medical aspects of the non-neuronal cholinergic system are published in the current special issue of *International Immunopharmacology*. Together with three previous symposium proceedings (ie, Life Sciences 2003, Vol. 72, No. 18–19; Life Sciences 2007, Vol. 80, No. 24–25; and Life Sciences 2012, Vol. 91, No. 21–22), the present issue of *International Immunopharmacology* provides the most comprehensive summary of the history and current progress in the development of the field of non-neuronal ACh.

The field of non-neuronal ACh has experienced an eventful past. ACh became famous as the first neurotransmitter to be discovered, and the Nobel Prize was awarded for this groundbreaking discovery in 1936 to Sir Henry Dale and Otto Loewi. At the same time, however, it was firmly established that ACh can also be isolated from non-neuronal sources such as spleen [1]. The following decades witnessed rapid progress in elucidating cholinergic neurotransmission, while the non-neuronal ACh was almost forgotten. Even in the 90s of the last century, just about 20 years ago, initiatives to investigate non-neuronal cholinergic mechanisms appeared to be provocative and faced skepticism. Nonetheless, step-by-step this fascinating topic has attracted more and more research groups that gathered in 2002 in San Francisco, USA, for a first international symposium on non-neuronal ACh. Follow-up meetings were held in Mainz, Germany (2007) and Groningen, The Netherlands (2011). Thus, it is no longer a matter of debate whether non-neuronal ACh is fact or fiction, and evidence of its eminent role in various aspects of human biology and pathology is rapidly growing, making it an attractive therapeutic target.

All components of the non-neuronal cholinergic system are expressed on epithelial, mesothelial and immune cells [2,3]. Epithelial ACh has been shown to be released into the lining fluid [4] and is involved in the regulation of ion- and water transport, mucociliary clearance and of the activity of immune cells. Mast cells, macrophages (*Mϕs*), dendritic cells (*DCs*), mononuclear lymphocytes (*MNLs*), neutrophils and eosinophils are endowed with muscarinic and nicotinic ACh receptors, *mAChRs* and *nAChRs*, which can be activated via autocrine or paracrine mechanisms. Thus, for example, ACh stimulates via M3 mAChR the release of proinflammatory cytokines from epithelial cells, Mϕs and smooth muscle fibers [5]. Importantly, the presence of ACh, the ACh synthesizing and degrading enzyme activities have also been detected in various plants [6–8]. Bamel et al. [9] have recently showed that nicotine as well as ACh promotes rooting in leaf explants of tomato, suggesting that ACh is acting as a natural growth regulator in plants.

At the molecular level, cellular ACh release mechanisms in non-neuronal cells can differ quite markedly from those seen at cholinergic synapses. Recently, experiments in cell-free systems identified OCTN1 (organic cation transporter novel 1) as a membrane-bound transporter

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Fig. 1. Participants of the Fourth International Symposium on Non-neuronal Acetylcholine in Giessen, Germany.

shuffling ACh across the membrane in a regulated, ATP-dependent fashion [10]. Data presented at the meeting convincingly showed that this mechanism also operates in mammalian cells [11]. Several studies addressed the fate of the ACh released by such mechanisms and the consequences of altering its extracellular stability. Exciting new data demonstrated that micro(*mi*)RNA links Toll-like receptor (*TLR*)9 activation to cholinergic signaling via targeting cholinesterases [12]. The authors have demonstrated differential reaction of intestinal cholinesterase-targeting miRNAs to distinct TLR9 challenges in mice exposed to both stress and canonical or alternative TLR9 oligonucleotide aptamer activators or blockers. TLR9 aptamers, but not stress, modulated cholinesterase-targeting miRNAs. These results implicated miRNA co-regulation in the intestinal alternative NF-κB pathway, and cholinergic signaling. The authors concluded that TLR9 aptamers may potentiate miRNA regulation that enhances cholinergic signaling and the resolution of inflammation, which would open new venues for treatment of bowel diseases. Enhancing cholinergic signaling by elevating ACh levels through reducing acetylcholinesterase (*AChE*) activity does not only result in such anti-inflammatory effects but also in increased production of pro-angiogenic factors, as exemplified in a muscular satellite cell line [13]. At the same time, these elevated ACh levels exert a positive feedback upon ACh synthesis via transcriptional upregulation of the ACh synthesizing enzyme, choline acetyltransferase (*ChAT*), demonstrating that myogenic cells themselves are a source of non-neuronal ACh involved in the regulation of angiogenesis. Key to the effects described here are elevated levels of extracellular ACh which were achieved by decreasing esterase activities. Surprising new findings revealed a novel mechanism of how this activity can be modulated. AChE and butyrylcholinesterase are pH-dependent with an optimum at pH above 7, whereas at pH values below 6 AChE is less active [14]. Thus, ACh is prevented from hydrolysis at such low pH values which occur at the surface of the human skin (pH around 5) and in several clinical conditions like metabolic acidosis, inflammation, fracture-related hematomas, cardiac ischemia and malignant tumors.

A comparative study of tissue specimens of larynx squamous cell carcinoma and adjacent non-cancerous tissue has demonstated that larynx cancer patients exhibit low ACh-degrading enzymatic activity, which correlates with a significantly shorter overall survival [15]. Differences in the mRNA levels of alternatively spliced AChE isoforms and molecular compositions were noted between glottic and supraglottic cancers. These results suggested that AChE activity and gene expression may be altered by cancer, and that the low AChE activity may be useful for predicting the outcome of patients with larynx squamous cell carcinoma.

New results reported by Alessandrini et al. [16] have demonstrated that activation of M2 mAChR inhibits growth and survival of human glioblastoma cancer stem cells. These results were obtained in experiments using both the pharmacological antagonism and the siRNA inhibition of M2 function in two glioblastoma cell lines derived from human biopsies. The authors concluded that M2 mAChR may represent a new therapeutic tool to control glioblastoma cancer stem cell growth and survival. In contrast, stimulation of the cholinergic receptors with carbachol on human breast adenocarcinoma cells amplified paclitaxel cytotoxicty, which could be abolished by atropine, thus implicating the mammary mAChRs [17]. Cytotoxicity was mainly due to the activation of nitric oxide synthase 1 and 3. Taken together, the two above-referenced studies suggested that while some mAChR subtypes, such as M2, are coupled to inhibition of cancer cell growth, the other subtypes may promote cancer cell survival and proliferation, which should have salient translational implications.

In addition to mAChRs, mammary epithelial cells are regulated through the nAChR-coupled pathway implicated in breast cancer [18], and it has been suggested that mammary nAChRs may become a specific target for novel anti-breast cancer therapies [19]. A recent study has demonstrated that both the non-malignant MCF10A and malignant MCF7 breast cells express α3, α5, α7, α9, α10, β1, β2, γ, δ and ε nAChR subunits and M1, M3, M4 and M5 mAChR subtypes, and that malignancy is associated with the appearance of α1, α4 and β4 nAChR subunits and M2 subtype, and overexpression of α7-, and α9-made nAChRs [20]. The carcinogenic tobacco nitrosamine NNK, which can activate nAChRs [21], upregulated ERK1/2 phosphorylation, stimulated expression of the gene encoding the tumor-promoter HGF, downregulated expression of the tumor suppressor gene CDKN2A, and induced tumorigenic transformation of MCF10A cells. Compared to the canonical nAChR antagonists, the nicotinergic peptide SLURP-1 showed the highest ability to abolish the nAChR-mediated effects of NNK in both cell-signaling and cell-transformation assays, and reversed many effects of NNK on gene expression. These results provided new insight into the molecular mechanisms of nAChR-mediated oncogenic effects of NNK, and demonstrated the ability of abolish or reverse these effects by SLURP-1. The latter is in agreement with previous reports about the anti-cancer activities of SLURP-1 [22,23], and suggests that this non-canonical nicotinergic ligand may become a novel pro-drug in the development of new treatments of both tobacco-related and non-related malignancies.

An exciting new finding is the involvement of non-neuronal ACh in neoplastic lung disease. In various lung cancer cell lines, it has been demonstrated that non-neuronal ACh stimulates proliferation of these tumor cells via auto- and paracrine pathways [24]. Ly-6 proteins represent a large family of proteins containing multiple cysteine residues (lynx1, lynx2, SLURP-1, SLURP-2) and resemble the snake three finger alpha toxins such as α-bungarotoxin. Thus, it is not surprising that members of the Ly-6 protein family can modulate nicotinic signaling. Most recently, Dr. Eliot Spindel's group has demonstrated that lynx1

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