RESPIRATORY SYNCYTIAL VIRUS INFECTION OF HUMAN RESPIRATORY EPITHELIAL CELLS ENHANCES BOTH MUSCARINIC AND β2-ADRENERGIC RECEPTOR GENE EXPRESSION

H. TSUTSUMI*, M. OHSAKI, K. SEKI, S. CHIBA

Department of Pediatrics, Sapporo Medical University School of Medicine, Chuoku S-1, W-16, Sapporo 060-8543, Japan

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Summary. – The possible changes in transcriptional activities of the M1 muscarinic acetylcholine receptor (mAChR) and β2-adrenergic receptor (AR) genes in respiratory syncytial virus (RSV)-infected human type 2 alveolar epithelial cells (A549 cells) were analyzed semiquantitatively by reverse transcription-polymerase chain reaction (RT-PCR). RSV enhanced M1 mAChR gene expression significantly at 4 hrs post infection (p.i.), and this enhancement persisted until 10 hrs, after peaking at 7 hrs. β2-AR gene expression also increased significantly as early as at 1 hr p.i. and persisted for more than 10 hrs. These transcriptional enhancements were observed in cells treated with live but not with inactivated virus. Our observations suggest that RSV infection of human respiratory epithelial cells may enhance the expression of both parasympathetic and sympathetic receptors. The upregulated M1 mAChR gene in virus-infected cells may correlate with temporal airway hyperresponsiveness in subjects with RSV or other respiratory virus infection. The enhanced β2-AR gene expression in peripheral lungs might explain the excessive mucus secretion observed during viral pneumonitis.

Key words: respiratory syncytial virus; human type 2 alveolar epithelial cells; muscarinic acetylcholine receptor; β2-adrenergic receptor

Airway infection with respiratory viruses including RSV exacerbate both asthma and chronic obstructive airway disease and also cause temporary airway hyperresponsiveness in previously normal individuals (Empey *et al.*, 1976; Schwarze *et al.*, 1997). The efferent, parasympathetic limb of the reflex arc has been implicated in this airway hyrperresponsiveness, since airway smooth muscle contraction in response to electrical stimulation of the vagus is potentiated during viral infections (Buckner *et al.*, 1985). The M1 mAChR facilitates neurotransmission and enhances cholinergic reflexes, and its localization was confirmed in parasympathetic ganglia, submucosal glands, and alveolar walls (Barnes, 1993).

β2-ARs regulate many lung functions including relaxation of airway and pulmonary vascular smooth muscle (Goldie *et al.*, 1982) and stimulation of mucus and surfactant secretion (Zaagsma *et al.*, 1983; Phipps *et al.*, 1982). β2-ARs are found in airway smooth muscle, epithelium submucosal glands, and type 2 alveolar cells (Dobbs and Mason, 1978; Castairs *et al.*, 1985; Hamid *et al.*, 1991).

The induction of several cytokines and chemokines, such as IL-1β, IL-6, IL-8, TNF-α, and RANTES, has been confirmed in RSV-infected human cells (Noah *et al.*, 1993; Tsutsumi *et al.*, 1996; Patel *et al.*, 1995; Olszewska-Pazdrak *et al.*, 1998). However, possible quantitative changes in autonomic receptors in virus-infected cells which might be involved in pathology of RSV disease have never been investigated.

*E-mail: tsutsumi@sapmed.ac.jp; fax: +81-11-611-0352.

Abbreviations: AR = adrenergic receptor; DMEM = Dulbecco's Modified Eagle's Medium; FCS = fetal calf serum; mAChR = muscarinic acetylcholine receptor; p.i. = post infection; RSV = respiratory syncytial virus; RT-PCR = reverse transcription-polymerase chain reaction

our experiments could have been caused by SMV without any interaction with TNV (*4*) or rhabdovirus. However, more than one virus may be involved in the term „mottle" as SMV is often accompanied by other viruses (*14*). No virus-like particles were observed in negatively stained preparations from healthy controls.

The only rhabdovirus so far described in strawberry is strawberry crinkle virus (SCV). Rhabdovirus particles of 69 ± 6 x 190–380 nm (*18*) and different sized bacilliform particles of 74 x 163 nm, 87 x 207 nm and 88 x 383 nm were described for SCV (*9*). Concerning the accuracy of measurement of rhabdovirus-like particles observed in our preparations and those mentioned above, the size of rhabdoviruses can be only approximate because of shrinkage taking place during dehydration of specimens for electron microscopy. Particles of these viruses readily deform and fragment *in vitro* unless pH and other conditions are closely controlled (*13*).

SCV is thought to be the causal agent of strawberry crinkle – one of the more important components of the viral diseases affecting strawberries world-wide because it reduces both yield and quality of the fruit. This cytoplasmic rhabdovirus often appears in combination with other strawberry viruses (viruses of strawberry mottle, vein banding, mild yellow-edge, and pallidosis) where it contributes additively to the disease severity (*7*). Although Hunter *et al.* (*9*) succeeded in preparing an antiserum against the G protein of SCV, and Schoen and Leone (*19*) prepared specific polyclonal mouse antisera against the M protein and the N plus Ns proteins, these could not be applied as diagnostic tools to replace the routine detection of strawberry propagative material by grafting to indicator plants. Within the major aphid-borne viruses, SMV and SCV still remain unidentified and largely uncharacterized (*20*). Very little is known about strains of SCV, its epidemiology, movement in nature or natural reservoirs (*9*).

Biological tests carried out by Erbenová (*3*) and Polák and Bezpalcová (*15*) indicated strawberry crinkle disease symptoms on graft-inoculated *F. vesca* clones, particularly in conjunction with mottle in former Czechoslovakia. Our finding is the first evidence of rhabdovirus-like particles in strawberry in the Czech Republic and the confirmation of complexity of strawberry mottle disease.

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