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Letter to the Editor

DsRNA disrupts airway epithelial barrier integrity through down-regulation of claudin members

Dear Editor,

Bronchial asthma is a pathological condition characterised by chronic allergic inflammation of the airway.[1] In recent years, it has been suggested that there is a close relationship between functional disturbances of airway epithelial cells and the development and exacerbation of asthma.[2] The airway is open to the external environment and serves as first line defence of the body against invading environmental factors.[3] While immunity is a self-defence system based on discrimination between self and non-self, the tight junctional barrier, which limits the entry of foreign antigens, can be referred to as the self–non-self boundary. Environmental factors, such as allergens, viruses and air pollutants cause repeated airway inflammation and direct epithelial injuries, which lead to disrupted barrier functions of the airway epithelium and excessive responsiveness to foreign substances. The E-cadherin-based cell adhesion apparatus of airway epithelial cells is important for the stabilisation of immune cells.[4] For example, house dust-induced damage to adherence junctions of the airway epithelium has been reported to promote TSLP production in airway epithelial cells.[5] In addition, E-cadherin and tight junction proteins are expressed in CD103-positive dendritic cells and are present between epithelial cells by homo binding to E-cadherin and tight junction proteins of epithelial cells.[6] These dendritic cells serve an immune surveillance function in a normal state; however, once epithelial injury is caused by inflammation, they undergo differentiation and migration and induce an immune response. The physical barrier function of the airway epithelium thus serves an immunoregulatory function and is believed to play a critical role in the development and progression of allergic inflammation.

Infection with RNA viruses, such as rhinovirus and respiratory syncytial virus, is a major cause of the exacerbation of asthma. At the time of RNA virus infection, double-stranded RNA (dsRNA) is inevitably produced during virus replication within host cells. dsRNA released outside cells is taken up by cells, recognised by Toll-like receptor 3 (TLR3) on endosomes, which induces the production of cytokines and chemokines. TLR3-mediated response of airway epithelial cells to dsRNA is an important element in the pathogenesis of allergic airway inflammation.[1,7]

In this study, we investigated the mechanism by which dsRNA destroys the airway epithelial cell barrier. Transformed human bronchial epithelial ($16HBE14_{o-}$, abbreviated as 16HBE) cells were kindly donated by Dr. Dieter C. Gruenert (Gene Therapy Center, Cardiovascular Research Institute, Department of Laboratory Medicine, University of California San Francisco, USA.). 16HBE cells were cultured in a Transwell chamber (Corning Costar, Lowell, MA, USA) for 24 h and for an additional 2 days in the presence of 1 or 10 μg/ml of polyinosinic–polycytidylic acid (poly-IC) (Sigma–Aldrich Corporation, St. Louis, MO, USA), a synthetic dsRNA. The epithelial barrier function was assessed by measuring the transepithelial electrical resistance (TER) with millicellTER (Millipore, Billerica, MA, USA). Permeability was measured using 4 kDa fluorescein isothiocyanate–dextran (FITC–dextran), and apparent permeability coefficients were calculated (Papp in cm/s).[8,9]

Poly-IC treatment of 16HBE cells decreased TER and increased FITC–dextran permeability (Fig. 1). It did not affect the proliferation or viability of 16HBE cells (data not shown). Transfection with TLR3-specific siRNA suppressed the poly-IC-induced decrease and increase in TER and FITC–dextran permeability, respectively, revealing the critical role of TLR3 activation in the epithelial barrier disruption (Fig. 1A). It has been reported that the intracellular TLR3 signal transduction involves pathways mediated by MyD88 and TRIF as adaptor molecules, through which transcription factors, such as NF-κB and IRF, are activated and cytokine production is induced. Next, we investigated the roles of MyD88 and TRIF in intracellular signalling pathways that are triggered by TLR3 activation and lead to the epithelial barrier disruption. The transfection with MyD88-specific siRNA significantly suppressed the TER in the condition with or without poly-IC treatment. The poly-IC-induced decrease in TER was not inhibited by transfection with TRIF-specific siRNA (Fig. 1B, C). These results suggest that constitutive expression and activation of Myd88 would be important for the stability of barrier function, and TRIF would play a crucial role on the TLR3-mediated barrier disruption.

Next, we used RT-PCR to analyse poly-IC-induced changes in mRNA expression of these molecules to determine the effects of TLR3 activation by dsRNA on the expression of tight junction and adherence junction molecules. After poly-IC treatment, with the exception of claudin-6 and -7, all claudin members that have been confirmed to be expressed in bronchial epithelial cells (claudin-1, -3, -4, -5, -6, -7, -9, -11, -16, -18 and -19) showed significant decrease in expression with a ≥50% decrease in cluadin-9 and claudin-19 (Fig. 2). In contrast, the expression of adherence junction protein E-cadherin and tight junction molecules occludin and ZO-1, and -2 did not decrease (Fig. 2). These results suggest that the epithelial barrier function was impaired through decreased expression of multiple claudin members as a consequence of the activation of the TLR3–TRIF-mediated signalling pathway.

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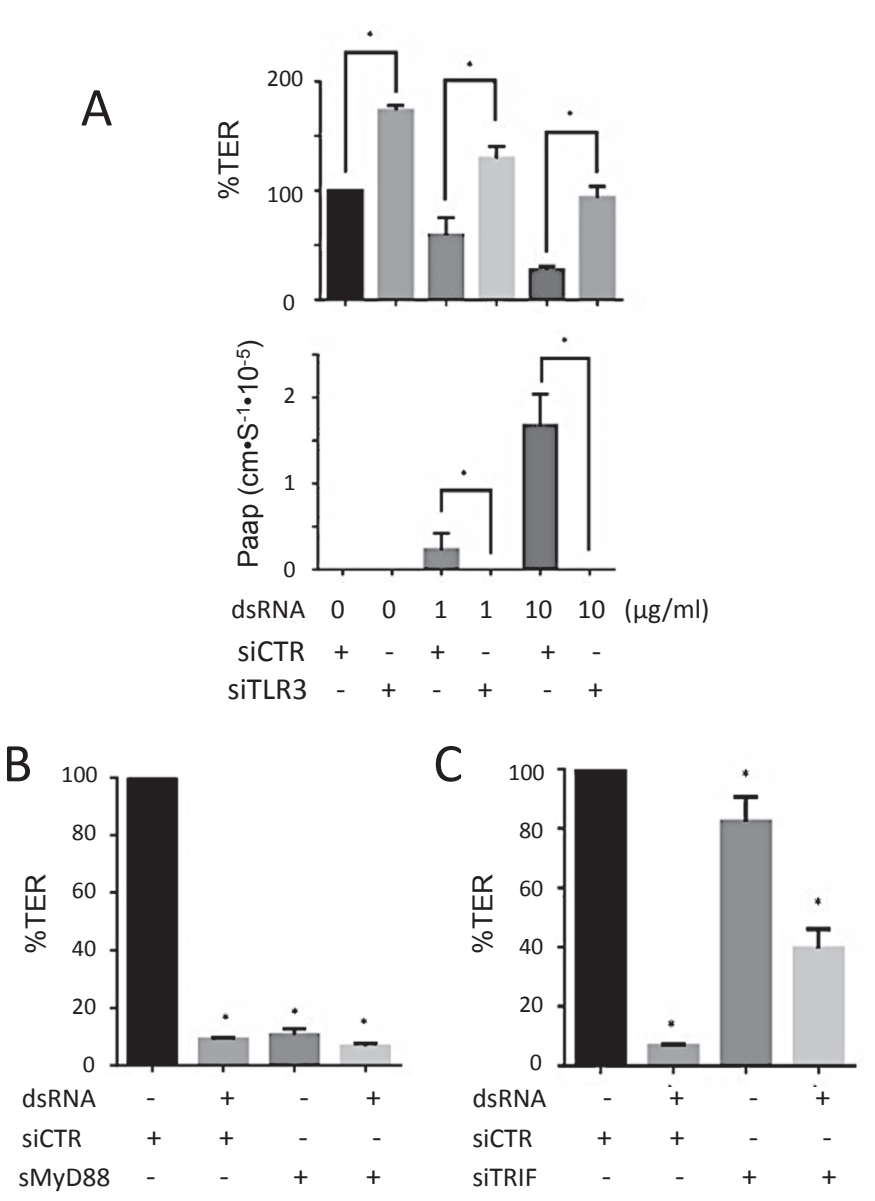

Fig. 1. Roles of TLR3, MyD88 and TRIF in poly-IC-induced barrier impairment. **(A, upper)** 16HBE cells transfected with control siRNA (siCTR) or TLR3-specific siRNA (siTLR3) were treated with poly-IC (1 or 10 μg/ml) and measured TER at 48 h after the poly-IC treatment. 16HBE cells transfected with siCTR or siTLR3 were measured TER at 48 h after the treatment. %TER represents percentage in reference to the siCTR-transfected cells without poly-IC treatment. **(A, bottom)** siCTR- and siTLR3-transfected cells were treated with poly-IC (1 or 10 μg/ml) and subjected to the FITC–dextran permeability assay at 48 h. The vertical axis represents apparent permeability coefficients (Paap).[8] **(B)** %TER of cells transfected with MyD88-specifc siRNA (siMyD88) at 48 h after the treatment with 10 μg/ml poly-IC. **(C)** %TER of cells transfected with TRIF-specific siRNA (siTRIF) after the treatment. Asterisks indicate a statistically significant difference ($P \leq 0.05$) compared to control.

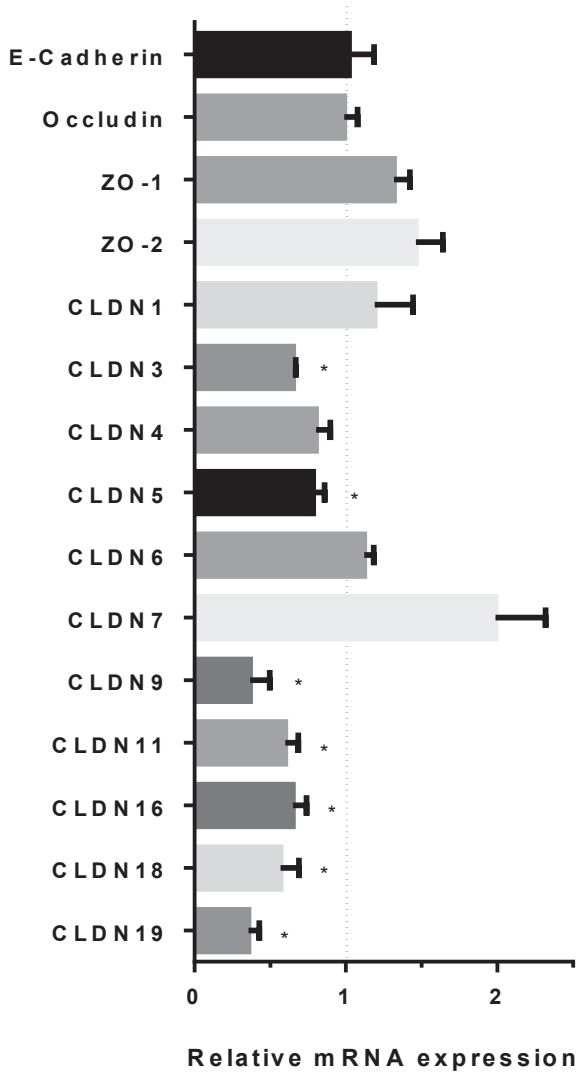

Fig. 2. Changes in expression levels of adherence junction and tight junction molecules after poly-IC treatment. RT-PCR analysis of E-cadherin, Zo-1 and -2 and claudin-1, -3, -4, -5, -6, -7, -9, -11, -16, -18 and -19 mRNA. Expression levels are shown as ratios of post-48-h-poly-IC-treatment to pre-treatment values. Asterisks indicate a statistically significant difference ($P \leq 0.05$) compared to control.

Based on previous studies, claudins can be divided into three functionally distinct groups. Claudin-1, -3, -4, -5, -8, -11, -14, -16 and -19 function to seal paracellular permeability, whereas claudin-2 and -10 enhance paracellular permeability. Claudin-7, -12, -15 and -16 have been reported to have both functions.[10] The dsRNA-induced decrease in the expression of claudin-3, -5, -11, -19 and other claudins in this study likely result in increased paracellular permeability. The mechanism by which TLR3–TRIF activation induced decreased expression of claudin members was not determined in the present study and will require further studies in the future.

In conclusion, the progression of bronchial asthma, which is closely related to RNA virus infection, has been suggested to be mechanistically related not only to the production of cytokines such as TSLP but also to the impaired barrier function of the epithelium due to decreased expression of claudin members, both of which are triggered by TLR3 activation by dsRNA.

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Conflict of interest

The authors have no conflict of interest to declare.

Yasuhiro Gon [a], Shuichiro Maruoka [a,*], Hiroyuki Kishi [c], Yutaka Kozu [a], Kazumichi Kuroda [b], Kenji Mizumura [a], Yasuyuki Nomura [c], Takeshi Oshima [c], Shu Hashimoto [a]

[a] *Division of Respiratory Medicine, Nihon University School of Medicine, Tokyo, Japan*
[b] *Division of Microbiology, Nihon University School of Medicine, Tokyo, Japan*
[c] *Division of Otolaryngology, Nihon University School of Medicine, Tokyo, Japan*

* Corresponding author. Division of Respiratory Medicine, Nihon University School of Medicine, 30-1 Ohyaguchi-Kamicho, Itabashi-ku, Tokyo 173-8610, Japan.
E-mail address: maruoka.shuichiro@nihon-u.ac.jp (S. Maruoka).

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