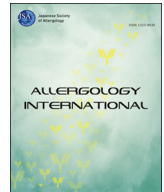




Contents lists available at ScienceDirect

Allergy International

journal homepage: <http://www.elsevier.com/locate/alit>

Letter to the Editor

CpG oligodeoxynucleotides enhance airway epithelial barrier integrity

Dear Editor,

Asthma is a common chronic disorder of the airways and involves a complex interaction of airflow obstruction, bronchial hyperresponsiveness, and airway remodeling.¹ In recent years, functional disturbances in the airway epithelial barrier have been suggested to be involved in the development and exacerbation of asthma.^{2,3} Environmental factors, such as allergens, viruses, and air pollutants, cause repeated airway inflammation and direct epithelial injuries, which lead to the disruption of the barrier functions of the airway epithelium. The functional disturbance in the airway epithelial cells as a physical barrier plays a critical role in the development and progression of allergic inflammation.^{2,3}

Previously, we reported that the activation of the Toll-like receptor 3 (TLR3)-mediated signaling pathway by double-stranded RNA (dsRNA) impairs the epithelial barrier function.^{4,5} RNA virus infection is believed to be involved in the pathogenesis of bronchial asthma.¹ TLR3 activation has been suggested to be mechanistically related not only to the production of cytokines, such as TSLP, IL-33, and IL-25, but also to the impaired barrier function.⁴ In addition, TLR9 agonists have demonstrated substantial potential as vaccine adjuvants for treating allergic diseases, including asthma. Commonly referred to as CpG oligodeoxynucleotides (ODN), TLR9 agonists directly induce the activation and maturation of plasmacytoid dendritic cells and enhance the differentiation of B cells into antibody-secreting plasma cells. Preclinical and early clinical data support the use of TLR9 agonists as vaccine adjuvants, which can enhance the responses to diverse antigens.⁶ The benefits of CpG ODN have been shown in multiple rodent and primate models of asthma⁷ and other allergic diseases, with encouraging results obtained in some early clinical trials.^{7–9}

In the present study, we investigated the effect of CpG ODN on the airway epithelial barrier integrity. Transformed human bronchial epithelial (16HBE140–, abbreviated as 16HBE) cells were kindly donated by Dr Dieter C. Gruenert (University of California, San Francisco, USA). Primary cultured normal bronchial epithelial (NBE) cells and 16HBE cells were cultured in a Transwell chamber (Corning Costar, Lowell, MA, USA) for 24 h and for an additional 3 days in the presence of 1.25–5 μ M CpG ODN (Sigma–Aldrich, St. Louis, MO, USA), a synthetic DNA. The epithelial barrier function was assessed by measuring the transepithelial electrical resistance (TER) using Millicell TER (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).^{10,11}

CpG ODN treatment did not affect the proliferation or viability of 16HBE cells (data not shown). There are three main types of CpG ODNs, which mimic the immunostimulatory activity of bacterial DNA, and are recognized as A-, B-, and C-class ODNs. Previous studies have reported that C-class ODNs possess the most efficient properties of B-cell activation with IFN- α secretion compared with A- and B-class ODNs.¹² The treatment of three types of CpG ODNs (ODN2216, ODN2006, and ODN2395) on 16HBE cells dose-dependently increased TER (Fig. 1A, upper) and decreased FITC–dextran permeability (Fig. 1A, lower). Transfection with TLR9-specific siRNA suppressed the poly-IC-dependent increase of TER, revealing the critical role of TLR9 activation in the CpG ODN-induced enhancement of epithelial barrier integrity (Fig. 1B).

In contrast to B cell activation by CpG ODNs, B-class CpG ODNs were the most effective for the development of barrier integrity compared with A- or C-class ODNs. The B-Class CpG ODNs contain one or more optimal hexameric CpG motifs and are stabilized against nuclease degradation by phosphorothioate modification.¹² However, in the present study, we could not clarify the exact molecular mechanism underlying such class-associated differences in the effects of ODNs.

To evaluate the role of CpG ODN on the epithelial barrier formation, we assessed the formation of adherens junctions (AJs) and tight junctions (TJs) in 16HBE cell monolayers by confocal immunofluorescence microscopy. After 3 days of treatment with CpG ODN, cells were fixed and stained with anti-E-cad-specific antibodies and anti-zonula occludens-1 (ZO-1) antibodies for visualizing AJs and TJs, respectively. ZO-1, occludin, and E-cadherin were strongly expressed and localized as junction proteins at the cell–cell contact sites on the apical surface of CpG ODN-treated cell monolayers compared with those in control cells (Fig. 2A). E-cadherin, but not ZO-1 or occludin, was also expressed and localized at the cell–cell contact sites at the bottom of control and CpG ODN-treated cell monolayers. The expression levels of E-cadherin were not different between control and CpG-treated cells (Fig. 2A). This result suggested that CpG ODN promotes the airway epithelial cell polarization.

Finally, we studied the effect of CpG ODNs on the primary cultured differentiated NBE cells cultured under an air–liquid interface condition. NBE cells basolaterally treated with 5 μ M CpG ODN (ODN2006) for 4 days demonstrated an increase in TER (Fig. 2 B) after subjecting to the air–liquid interface condition.

In conclusion, the progression of bronchial asthma has been suggested to be mechanistically related to the impaired barrier

Peer review under responsibility of Japanese Society of Allergy.

<http://dx.doi.org/10.1016/j.alit.2017.05.002>1323–8930/Copyright © 2017, Japanese Society of Allergy. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

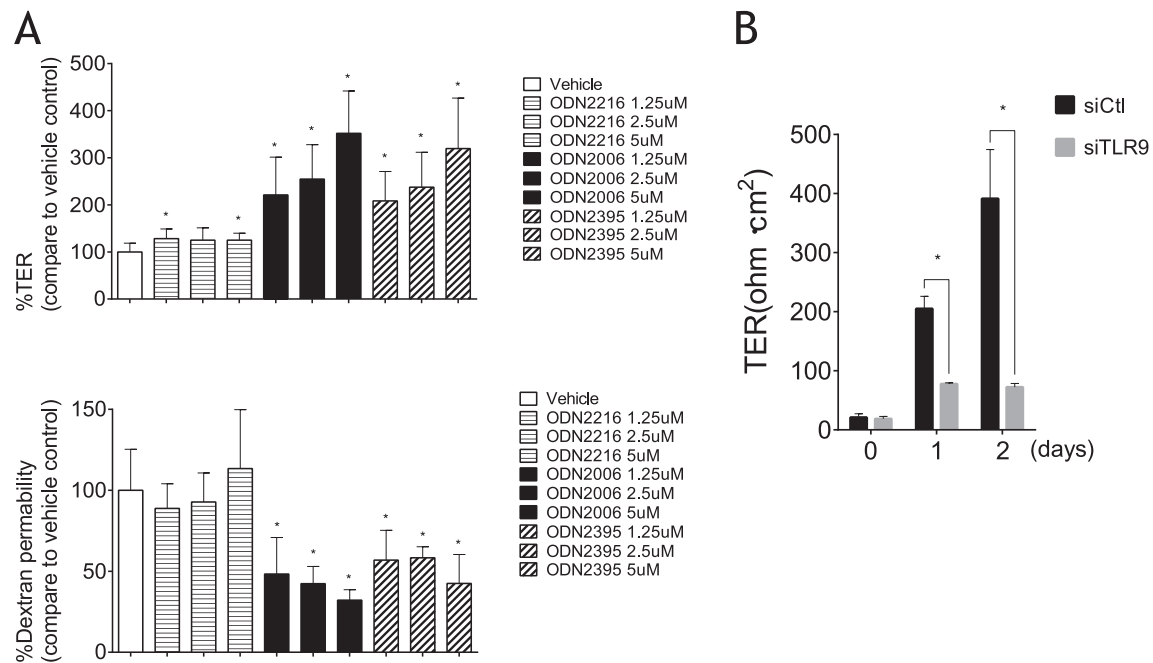


Fig. 1. Roles of CpG oligodeoxynucleotides (ODNs) on the epithelial barrier integrity. **(A)** Upper: human bronchial epithelial (16HBE) cells treated with control [phosphate buffered saline (PBS)] or the indicated dose of CpG ODNs (ODN2216, ODN2006, or ODN2395), and transepithelial electrical resistance (TER) was measured 48 h after the treatment. %TER represents percentage in reference to the control cells. **(A)** Lower: 16HBE cells treated with control (PBS) or the indicated dose of CpG ODNs (ODN2216, ODN2006, or ODN2395), and TER was measured 48 h after the treatment. The vertical axis represents apparent permeability coefficients (Paap).¹⁰ **(B)** %TER of cells transfected with Toll-like receptor 9 (TLR9)-specific small interfering RNA (siRNA) (siTLR9) at 48 h after the treatment with 5 μ M CpG ODNs. Results are mean $\pm$ standard deviation (SD) values. Asterisks indicate a statistically significant difference ($P \leq 0.05$) compared with the control.

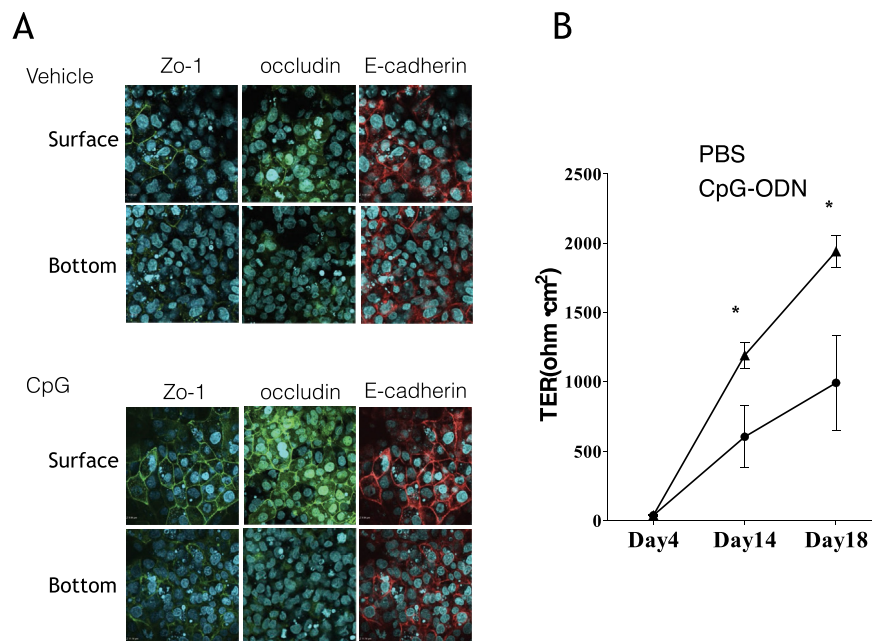


Fig. 2. **(A)** Changes in the expression of adherens junction and tight junction molecules after CpG oligodeoxynucleotides (ODN) treatment. Immunocytochemical analysis of E-cadherin (E-cad) and zonula occludens-1 (ZO-1) in human bronchial epithelial (16HBE) cells. 16HBE cells were treated with 5 μ M CpG ODN (ODN2006). After 48 h, cells were subjected to immunocytochemical analysis using antibodies specific to ZO-1 (green), occludin (green), or E-cad (red). The images represent data from three independent experiments. **(B)** Effect of CpG ODN (ODN2006) on the transepithelial electrical resistance (TER) of differentiated primary bronchial epithelial cells. Primary cultured bronchial epithelial cells were cultured in a Transwell chamber with or without CpG ODN (ODN2006). Subsequently, TER was measured at days 4, 14, and 18 after subjecting the cells to air–liquid interface (ALI) condition. TER values are expressed as mean $\pm$ standard deviation (SD). Asterisks indicate a statistically significant difference ($P \leq 0.05$) compared with the control.

function of the epithelium. Our data suggested that CpG DNA treatment can be useful for treating asthma by restoring the impaired epithelial barrier.

Acknowledgment

The results in this study have been already published as a Japanese patent information (P130008649), January 2014 (applied in July 2012). This work was supported by the following grants: Grants-in-Aid for Scientific Research, (C) (2479210) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT). The Strategic Research Base Development Program for Private Universities subsidized by MEXT (S1091023, since 2010), and the Nihon University President's Grant for Multidisciplinary Studies.

Conflict of interest

The authors have no conflict of interest to declare.

Yasuhiro Gon ^a, Sotaro Shikano ^a, Shuichiro Maruoka ^{a,*}, Kenji Mizumura ^a, Yutaka Kozu ^a, Kazumichi Kuroda ^b, Eriko Tsuboi ^a, Ikuko Takeshita ^a, Hiroyuki Kishi ^c, 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. (S. Maruoka).

E-mail address: maruoka.shuichiro@nihon-u.ac.jp (S. Maruoka).

References

1. Hashimoto S, Matsumoto K, Gon Y, Ichiwata T, Takahashi N, Kobayashi T. Viral infection in asthma. *Allergol Int* 2008;**57**:21–31.
2. Georas SN, Rezaee F. Epithelial barrier function: at the front line of asthma immunology and allergic airway inflammation. *J Allergy Clin Immunol* 2014;**134**:509–20.
3. Nawijn MC, Hackett TL, Postma DS, van Oosterhout AJ, Heijink IH. E-cadherin: gatekeeper of airway mucosa and allergic sensitization. *Trends Immunol* 2011;**32**:248–55.
4. Gon Y, Maruoka S, Kishi H, Kozu Y, Kuroda K, Mizumura K, et al. DsRNA disrupts airway epithelial barrier integrity through down-regulation of claudin members. *Allergol Int* 2016;**65**(Suppl):S56–8.
5. Gon Y. Toll-like receptors and airway inflammation. *Allergol Int* 2008;**57**:33–7.
6. Kumagai Y, Takeuchi O, Akira S. TLR9 as a key receptor for the recognition of DNA. *Adv Drug Deliv Rev* 2008;**60**:795–804.
7. Jain VV, Kitagaki K, Kline JN. CpG DNA and immunotherapy of allergic airway diseases. *Clin Exp Allergy* 2003;**33**:1330–5.
8. Casale TB, Cole J, Beck E, Vogelmeier CF, Willers J, Lassen C, et al. CYT003, a TLR9 agonist, in persistent allergic asthma – a randomized placebo-controlled Phase 2b study. *Allergy* 2015;**70**:1160–8.
9. Beeh KM, Kanniss F, Wagner F, Schilder C, Naudts I, Hamann-Haenni A, et al. The novel TLR-9 agonist QbG10 shows clinical efficacy in persistent allergic asthma. *J Allergy Clin Immunol* 2013;**131**:866–74.
10. Kozu Y, Gon Y, Maruoka S, Kazumichi K, Sekiyama A, Kishi H, et al. Protocadherin-1 is a glucocorticoid-responsive critical regulator of airway epithelial barrier function. *BMC Pulm Med* 2015;**15**:80.
11. Gon Y, Matsumoto K, Terakado M, Sekiyama A, Maruoka S, Takeshita I, et al. Heregulin activation of ErbB2/ErbB3 signaling potentiates the integrity of airway epithelial barrier. *Exp Cell Res* 2011;**317**:1947–53.
12. Vollmer J, Weeratna R, Payette P, Jurk M, Schetter C, Laucht M, et al. Characterization of three CpG oligodeoxynucleotide classes with distinct immunostimulatory activities. *Eur J Immunol* 2004;**34**:251–62.

Received 16 January 2017

Received in revised form 12 April 2017

Accepted 21 April 2017

Available online xxx