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Abiotic stress of ambient cold temperature regulates the host receptivity to pathogens by cell surfaced sialic acids

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ABSTRACT

Ambient cold temperature, as an abiotic stress, regulates the survival, stability, transmission, and infection of pathogens. However, the effect of cold temperature on the host receptivity to the pathogens has not been fully studied. In this study, the expression of terminal α-2,3- and α-2,6-sialic acids were increased in murine lung tissues, especially bronchial epithelium, by exposure to cold condition. The expression of several sialyltransferases were also increased by exposure to cold temperature. Furthermore, in human bronchial epithelial BEAS-2B cells, the expressions of α-2,3- and α-2,6-sialic acids, and mRNA levels of sialyltransferases were increased in the low temperature condition at 33 °C. On the other hand, the treatment of Lith-Gly, a sialyltransferase inhibitor, blocked the cold-induced expression of sialic acids on surface of BEAS-2B cells. The binding of influenza H1N1 hemagglutinin (HA) toward BEAS-2B cells cultured at low temperature condition was increased, compared to 37 °C. In contrast, the cold-increased HA binding was blocked by treatment of lithocholicglycine and sialyl-*N*-acetyl-*D*-lactosamines harboring α-2,3- and α-2,6-sialyl motive. These results suggest that the host receptivity to virus at cold temperature results from the expressions of α-2,3- and α-2,6-sialic acids through the regulation of sialyltransferase expression.

1. Introduction

The relationship between environmental cold temperature and susceptibility of respiratory infection is generally accepted [1,2]. Several recent studies suggested that Cold and dry weather may be a casual factor in transmission and mortality of influenza [3–5]. Especially, the cold temperature has been regarded as an environmental factor regulating the survival, stability, and transmission of pathogens [5–7]. With respect to severity of inflammation, several studies showed that ambient cold temperature is related with increased production of inflammatory cytokines, such as interleukin (IL)-1β, IL-6, IL-12, and IL-17 [8–10]. However, in case of influenza infection, innate immune response is not affected by cold temperature as previously demonstrated [5]. Thus, we focused on another hypothesis that cold temperature may affect the host receptivity to the pathogens.

Many important pathogenic viruses, including influenza, use the terminal sialic acid structures linked to glycoproteins and glycolipids as a receptor for entrance to the host cells [11,12]. The binding sites to sialic acid are often highly conserved in homologous viruses and structural diversities of sialic acid epitopes are also specially recognized by different viruses [12]. For example, the avian and human influenza viruses bind to different sialic acids linked to galactose by an α-2,3 linkage and α-2,6 linkage, respectively [13,14]. Previously, the influence of ambient cold temperature on the contents and composition of gangliosides was studied in the brain of some vertebrates including fish, hamster, pig, and rat [15–17]. However, the effect of cold temperature on the expression of sialic acid epitope, as a receptor for viral infection, was not investigated.

In this study, we examined the expression and distribution of α-

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2,3 and α-2,6 sialic acid epitopes in the mice lung tissues exposed to cold temperature. In addition, the expression of sialic acid epitopes on the surface of human bronchial cells was also determined. By inhibiting sialyltransferase activity, the relationship between expression of sialic acid epitope and hemagglutinin binding was analyzed. Given importance of Sia-epitopes to the viral infection, these results may be useful for understanding the molecular action of noxious cold on the susceptibility to viral infection.

2. Materials and methods

2.1. Materials

Biotinylated lectins recognizing α-2,3- and α-2,6-sialic acid epitopes, *Maackia amurensis* lectin II (MAL II) and *Sambucus nigra* agglutinin (SNA), respectively, were purchased from Vector Labs (Burlingame, CA, USA). Recombinant C-terminal His-tagged hemagglutinin (HA) deduced from influenza A H1N1 (A/Puerto Rico/8/34) was supplied by Sino Biologicals Inc. (Beijing, China). Antibody against H1N1 HA was obtained from Takara Bio Inc. (Shiga, Japan). Horseradish peroxidase (HRP)-conjugated streptavidin, Alexa Fluor488-conjugated streptavidin, and Alexa Fluor488-conjugated mouse IgG were purchased from Invitrogen (Waltham, MA, USA). An inhibitor against sialyltransferase activity, lithocholylglycine (Lith-Gly) was purchased from Santa-Cruz Biotechnology (Santa Cruz, CA, USA). Alpha-2,3- and α-2,6-Sialyl-*N*-Acetyl-D-lactosamine (3-SLN and 6-SLN) were purchased from Carbosynth Ltd. (Berkshire, UK). All the other chemicals and reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise indicated.

2.2. Animals and exposure to ambient cold

Male C57BL/6 mice (seven weeks old, weighing about 20–24 g) were purchased from Orient Bio Inc. (Sungnam, Korea). The animals were freely accessed to a standard diet with drinking water prior to the experiment. All mice were kept in laboratory cage rack systems maintained at constant temperature (22 ± 1 °C or 4 ± 1 °C), humidity (50 ± 5%). The rooms maintained at 12 h dark/light cycles. Cold stress was induced by long term exposure to 4 °C for 8 h each day for 14 days (Fig. 1A), according to previous reports with some modifications [5,18,19]. The mice were sacrificed by euthanizing with CO_2 gas immediately after last exposure to cold. Bilateral bronchoalveolar lavage (BAL) was performed for our previous study [9] and lung tissues were collected. All experimental procedures followed the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health of Korea, and all the experiments were approved by the Institutional Animal Care and Use Committee of Pusan National University (protocol PNU-2015-0856; Busan, Korea).

2.3. Lectin blot analysis

The lung tissues and cultured cells were homogenized in ice-chilled 1% NP-40 lysis buffer containing 150 mM sodium chloride (NaCl), 10 mM HEPES, 1% NP-40, 5 mM sodium pyrophosphate ($Na_4O_7P_2$), 5 mM sodium fluoride (NaF), 2 mM sodium orthovanadate (Na_3VO_4), and a protease inhibitor cocktail tablet (Roche, Mannheim, Germany). The homogenized samples were centrifuged at 15,000 rpm at 4 °C for 15 min. The supernatants were collected and stored at −80 °C before use for the lectin blot analysis. The protein amounts were measured using the Bradford method (Bio-Rad protein assay, Bio-Rad Laboratories, Hercules, CA, USA). Equal amounts (30 μg) of proteins were separated using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and transferred onto a nitrocellulose membrane (Bio-Rad Laboratories). The blots were blocked for 1 h Carbo-Free blocking Solution (Vector Labs) prior to incubation with biotinylated MAL II and SNA at 4 °C overnight. After incubating the blots with the HRP-conjugated streptavidin at room temperature for 1 h, the bands of interest were visualized using chemiluminescence (ECL Plus; Invitrogen). The band intensities obtained from the western blot analysis were quantified using the ImageJ software (NIH, Bethesda, MA, USA).

2.4. Lectin immunohistochemistry

The lung tissues were 10% formalin fixed and washing in tap water. The standard portion of lung tissues were 10% formalin fixed, paraffin-embedded tissue sections of 3 μm thickness were deparaffinized and rehydrated. Heat-induced epitope retrieval was performed using citrate buffer (pH 6.0) in pressure cooked for 40 min. Endogenous peroxidase activity was blocked using 3% H_2O_2 in methanol for 10 min. Tissue sections were then incubated with diluted MAL II or SNA (1:100) for 120 min at room temperature in a humidified chamber. After washing 3 times with TBS, tissue slides were incubated with VECTORSTAIN ABC Kit as per the manufacturer's instructions (Vector Labs). Tissue sections were incubated with 3,3′-diaminobenzidine/H_2O_2 (Dako) for color development and counter-stained with mayer's hematoxylin.

2.5. Lectin FACS analysis

The BEAS-2B cells were fixed with 3.7% formalin and washed twice with 1 × PBS. The cells were blocked with Carbo-Free blocking Solution for 1 h and were incubated with diluted biotinylated lectins (1:100), MAL II or SNA, for 120 min at 4 °C. After incubation, the cells were reacted with Alexa Fluor488-conjugated streptavidin (1:5000) at 4 °C for 1 h. Then, the samples were examined on a BD FACSCANTO II (BD Biosciences, Franklin Lakes, NJ, USA), measuring excitation/emission at 494/525 nm.

2.6. HA binding assay

The BEAS-2B cells were fixed with 3.7% formalin, washed twice with 1 × PBS, and blocked with Carbo-Free blocking Solution for 1 h. The cells were incubated with influenza H1N1 (A/Puerto Rico/8/34) HA (2 μg/mL) at room temperature for 1 h. Then, the samples were reacted with antibody against H1N1 HA (1 μg/mL) for 1 h. After the reaction, the samples were incubated with Alexa Fluor488-conjugated anti-mouse IgG for 40 min the samples were examined on a BD FACSCANTO II, measuring excitation/emission at 494/525 nm.

2.7. Confocal microscopic observation

The BEAS-2B cells were seed on glass coverslips and cultured at 37 °C or 33 °C for 72 h. The cells were fixed with 3.7% formaldehyde in 1 × PBS. After incubating with Carbo-free blocking solution for 1 h, the cells were incubated with MAL II (1:100) or SNA (1:100) for 2 h, followed by incubation with Alexa Fluor488-conjugated streptavidin (1:5000) for 1 h. After washing with ice-chilled 1 × PBS, the samples were mounted on glass slides using VECTASHIELD mounting solution. Fluorescent images were obtained using a ZEISS LSM700 laser scanning confocal microscope (Carl Zeiss, Oberkochen, Germany).

2.8. Statistical analysis

The data from lectin blot analysis and RT-PCR were calculated as percentage compared to the control, and are expressed as the