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Exposure to low temperatures suppresses the production of B-cell activating factor via TLR3 in BEAS-2B cells

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ABSTRACT

Acute viral respiratory tract infections (RTIs) are commonly associated with cold weather; however, the mechanism behind this is still unclear. Secretory IgA (sIgA) mainly contributes to the immune response against pathogenic microorganisms in the respiratory tract. Certain pathogen-associated molecular patterns (PAMPs) induce the expression of B-cell activating factor (BAFF) in epithelial cells, macrophages, and dendritic cells. BAFF transforms B cells into plasma cells, which leads to the mass production of immunoglobulins, including IgA, on the mucosal epithelium. However, no studies have described the relationship between cold exposure and BAFF and/or sIgA in RTI. The aim of our study was to determine this relationship *in vitro* by investigating the effect of low temperature on BAFF production by BEAS-2B cells after the addition of toll-like receptor (TLR) ligands. We showed stimulation of polyinosinic:polycytidylic acid (poly I:C), which led BEAS-2B to produce interferon (IFN)-β. IFN-β itself induced BEAS-2B cells to produce BAFF. Janus kinase inhibitor I decreased the amount of BAFF produced in BEAS-2B cells upon stimulation with IFN-β and poly I:C. Significantly less BAFF was produced post-poly I:C stimulation in low-temperature conditions than in normal-temperature conditions (mean ± SD: 41.2 ± 23.3 [33 °C] vs. 138.3 ± 7.1 pg/mL [37 °C], $P = 0.05$). However, the low-temperature condition itself was not cytotoxic. The stimulation of poly I:C produced BAFF from BEAS2B cells via IFN-β production and the JAK/signal transducer and activator of transcription pathway played an important role in BAFF production in BEAS-2B cells. Cold exposure reduced BAFF production by BEAS2B cells after stimulation with the TLR3 ligand. Cold exposure may, therefore, suppress the production of BAFF, resulting in the inhibition of IgA secretion in the bronchial epithelium, which explains the increased frequency of RTIs in cold weather.

1. Introduction

The development of acute upper respiratory tract infections (URTIs), including the common cold, is related to exposure to low temperatures. In fact, the association between exposure to low temperatures and infection may be responsible for the origin of the term “common cold.” Indeed, a previous report has shown that low temperatures can increase the risk of transmission of pathogens such as influenza viruses and human rhinoviruses [1].

Several possible mechanisms associated with exposure to low temperatures may increase the risk of URTIs. In terms of systemic responses, it has been reported that low temperatures can lead to increased white blood cell counts, granulocyte counts, and plasma inflammatory cytokine, such as interleukin-6 (IL-6), concentrations. Moreover, the activity of natural killer (NK) cells is retained even after exposure to cold air [2]. These alterations may be influenced by exercise and caused by stress hormones. In another study, a small and nonsignificant increase in NK cell activity after cold exposure has been reported [3]. In terms of the local immune responses, Salman et al. reported that the phagocytic capacity of macrophages in rats is very sensitive to temperature change, and a temperature drop of only 1.5 °C is sufficient to inhibit phagocytic activity [4]. It has also been reported that the reflex vasoconstrictor response in the epithelium of the nasal cavity and upper respiratory tract, triggered by surface cooling, is maintained for longer periods of time in individuals who are more prone to developing URTIs. Therefore, vasoconstriction can contribute to the development of characteristic symptoms of a common cold, such as decreased blood flow and white blood cell supply [5]. Walsh et al. demonstrated that salivary IgA levels following exercise at cold temperatures are decreased [6]. In terms of viral replication, Foxman et al. showed that some respiratory viruses,

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including rhinoviruses, replicate more efficiently at 33 °C, which is below the normal body temperature [7].

Given the local immune system, secretory IgA (sIgA) is currently recognized as a major effector of mucosal immunity. IgA mainly contributes to the fight against infections through antigen excretion, intracellular neutralization, and immune exclusion. IgA is abundantly secreted on the mucosal surface of plasma cells. IgA class switching is a key process by which B cells differentiate and acquire IgA-producing capacity, and both T cell-dependent (TD) and T cell-independent (TI) pathways are involved. Of these two pathways, the TD pathway takes at least 5 days. This time gap could be fatal regarding microbial invasion in the mucous membrane [8]. However, via the TI pathway, IgA can be produced more rapidly than through the TD pathway. IgA production by the TI pathway requires the activity of B-cell activating factor (BAFF), a cytokine that is a member of the tumor necrosis factor family [9]. BAFF is an important factor in the maturation and survival of B cells and is produced by various cells such as epithelial cells and dendritic cells [10]. Previous studies have demonstrated that BAFF and its homolog, a proliferation-inducing ligand that is usually named "APRIL," can cause IgA class switching through the TI pathway. Recently, McNamara et al. showed that respiratory syncytial virus infection results in increased BAFF expression in the airways [11].

One study carried out to understand the relationship between sIgA and exposure to low temperatures has shown that salivary IgA levels following exercise at cold temperatures are decreased in human athletes [12]. As no studies have discerned the relationship between cold exposure and BAFF as an inducer of sIgA in the respiratory mucosa, the aim of our study was to investigate this relationship *in vitro*.

2. Materials and methods

2.1. Cell culture and reagents

BEAS-2B cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA, USA) supplemented with 4 mM glutamine (Gibco Invitrogen, Carlsbad, CA, USA), 10% (v/v) fetal calf serum, 100 units/mL penicillin G, and 100 μg/mL streptomycin (MP Biomedicals, Irvine, CA, USA) at 37 °C in a humidified atmosphere with 5% CO_2. Reagents for cell stimulation, including the toll-like receptor (TLR) ligands lipoteichoic acid (LTA), polyinosinic: polycytidylic acid (poly I:C), lipopolysaccharide (LPS), and flagellin were purchased commercially (LTA, LPS, and flagellin were purchased from InvivoGen, San Diego, CA, USA; poly I:C was purchased from R&D Systems, Minneapolis, MN, USA). Recombinant human IFN-β was purchased from Abcam (Cambridge, UK). The IFN-β signaling pathway inhibitor, Janus kinase (JAK) inhibitor I, was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The TLR3-neutralizing antibody was purchased from Abcam.

2.2. Enzyme-linked immunosorbent assay (ELISA)

An ELISA was performed in the same manner as previously described [13]. After stimulating cells with TLR ligands or human IFN-β, cell culture supernatants were collected and stored at −80 °C for performing ELISA. TLR ligand-induced BAFF or IFN-β production was assayed using the human BAFF ELISA Kit (R&D Systems) or human IFN-β ELISA Kit (Kamakura Techno-Science, Kamakura, Japan) according to the manufacturers' instructions.

2.3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis

SDS-PAGE was performed as described by Laemmli [14]. The gels were transferred onto nitrocellulose membranes in the presence of an electric current for immunoblotting. The membrane was blocked in a blocking buffer containing 3% (w/v) bovine serum albumin (Millipore, Billerica, MA, USA) for 1 h. The membrane was then incubated with a primary antibody and subsequently with a horseradish peroxidase (HRP)-labeled anti-immunoglobulin antibody. Antibodies against β-actin (Santa Cruz Biotechnology) and type 1 IFN receptor, IFNAR1 (EP899Y) (Abcam) were used. The protein bands were visualized using Immobilon Western Chemiluminescent HRP Substrate (Millipore).

2.4. TLR3 stimulation of bronchial epithelial cells and its effect on BAFF production

BEAS-2B cells were grown to 90% confluency before adding TLR ligands (LTA; 100 μg/mL, LPS; 10 μg/mL, flagellin; 100 ng/mL, and poly I:C; 100 μg/mL) to the culture media. Each TLR ligand was added individually for 72 h, and the amount of BAFF in the culture media was measured by ELISA. To assess the role of TLR3 in the induction of BAFF production, cells were treated with an anti-TLR3 neutralizing antibody or a human IgG isotype control. The method of using a neutralizing antibody was performed in the same manner as previously shown [13]. The treated cells were then stimulated with poly I:C or vehicle, and BAFF production was assayed using the human BAFF ELISA Kit.

2.5. Role of poly I:C-induced IFN-β production in BAFF production

BEAS-2B cells were stimulated with poly I:C or vehicle and IFN-β production was assayed using the human IFN-β ELISA Kit.

2.6. Effect of temperature on the production of BAFF via TLR3 stimulation and type 1 IFN receptor expression in BEAS-2B cells

BEAS-2B cells were maintained at 37 °C or 33 °C for 3 days. Cells grown under both conditions were stimulated with poly I:C, and the amount of BAFF in the culture media was measured by ELISA.

We next investigated the type 1 IFN receptor expression of BEAS-2B cells in normal and low-temperature settings. BEAS-2B cells were maintained at 37 °C or 33 °C for 3 days, and the expression level of IFNAR1 was measured by SDS-PAGE and western blotting.

3. Results

3.1. TLR3 stimulation can induce BAFF production by bronchial epithelial cells

Poly I:C, but not the other TLR ligands, induced the production of BAFF by BEAS-2B cells (Fig. 1A). Stimulation with poly I:C induced BAFF production in the presence of an isotype control human IgG. However, poly I:C-induced BAFF production was significantly inhibited in BEAS-2B cells pretreated with the anti-TLR3 neutralizing antibody (Fig. 1B).

3.2. Poly I:C induction promoted IFN-β production

Stimulation with poly I:C promoted IFN-β production (Fig. 1C). Moreover, IFN-β itself induced BEAS-2B cells to produce BAFF. JAK inhibitor I, an inhibitor of the IFN-β signaling pathway, decreased the amount of BAFF produced in BEAS-2B cells upon stimulation with IFN-β and poly I:C (Fig. 1D and E).

3.3. Low temperatures reduce the production of BAFF

The amount of BAFF produced by cells grown under low-temperature conditions after poly I:C stimulation was significantly lower than that produced by cells grown at 37 °C (mean ± standard deviation: 41.2 ± 23.3 pg/mL at 33 °C versus 138.3 ± 7.1 pg/mL at 37 °C; $P = 0.05$) (Fig. 2A). However, the amount of IFN-β produced by cells grown under low-temperature conditions after stimulation with

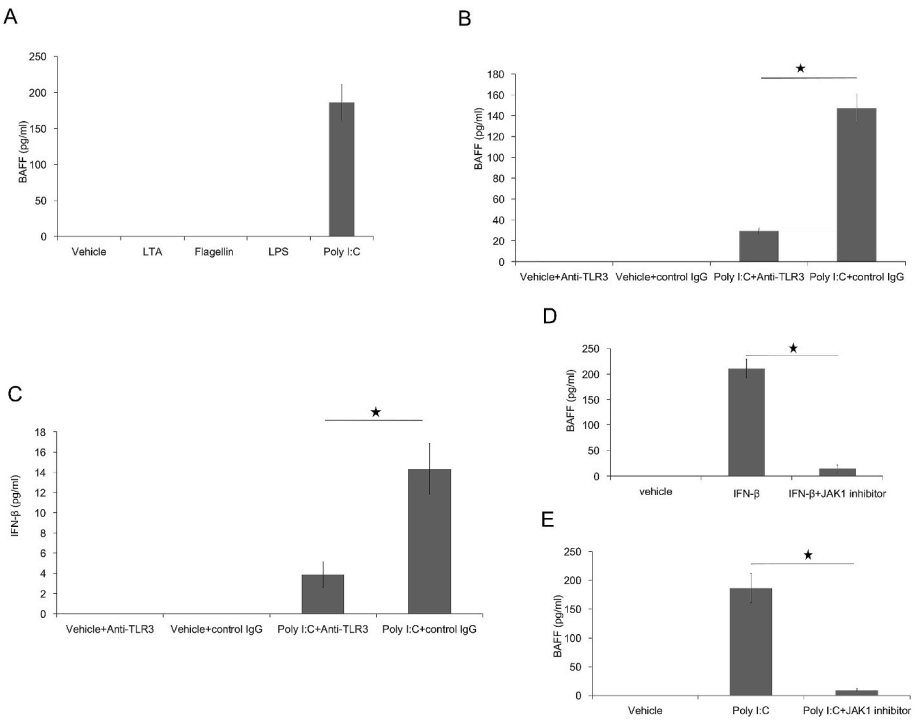

Fig. 1. Poly I:C induces the production of BAFF in BEAS-2B cells in a TLR3- and IFN-β-dependent fashion. (A) BAFF concentration was measured in each culture medium 72 h after treatment with vehicle, LTA (100 μg/mL), flagellin (100 ng/mL), LPS (10 μg/mL), or poly I:C (100 μg/mL). Only poly I:C induced the production of BAFF (186.4 ± 25.4 pg/mL). (B) One hour before stimulation with poly I:C, a TLR3-neutralizing antibody or isotype control human IgG was added to the culture medium. After poly I:C stimulation, we measured BAFF production as in (A). There was a significant difference in BAFF production between BEAS-2B cells treated with an anti-TLR3 antibody and cells treated with isotype control human IgG. (C) One hour before stimulation with poly I:C, a TLR3-neutralizing antibody or isotype control human IgG was added to the culture medium, and IFN-β production was measured 24 h after poly I:C stimulation. There was a significant difference in the IFN-β production between BEAS-2B cells treated with an anti-TLR3 antibody and cells treated with isotype control human IgG. (D) One hour before stimulation with IFN-β, JAK inhibitor I or vehicle was added to the culture medium, and BAFF concentration was measured 48 h after IFN-β stimulation. There was a significant difference in the BAFF production between BEAS-2B cells treated with JAK inhibitor I and cells treated with vehicle alone. (E) One hour before stimulation with poly I:C, JAK inhibitor I or vehicle was added to the culture medium, and BAFF concentration was measured 72 h after poly I:C stimulation. There was a significant difference in the BAFF production between BEAS-2B cells treated with JAK inhibitor I and cells treated with vehicle alone.

poly I:C was significantly higher than that produced by cells grown at 37 °C (Fig. 2B). We also assessed whether IFN-β could induce BAFF production in BEAS-2B cells in a dose-dependent manner in low-temperature settings. There was no dose-dependency in BAFF production upon the stimulation of cells with IFN-β in low-temperature settings (Fig. 2C).

The expression of IFNAR1 was lower in cells maintained under low-temperature conditions than in those maintained under 37 °C (Fig. 2D).

4. Discussion

We showed that BEAS-2B cells could produce BAFF upon stimulation with poly I:C as a molecular pattern associated with viral infection. These results showed that the airway epithelium can efficiently produce

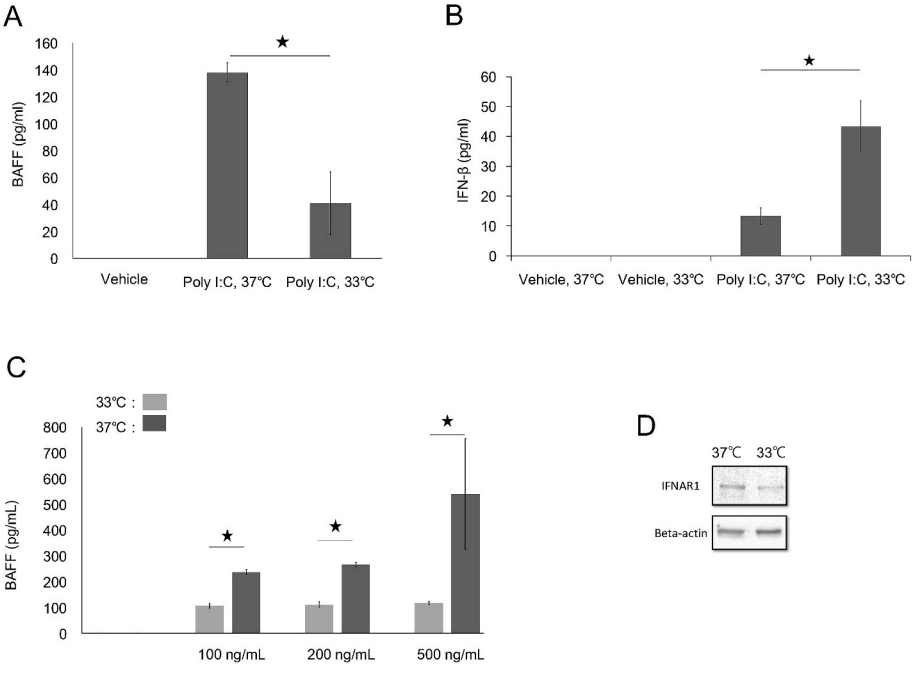

Fig. 2. Low temperatures can inhibit BAFF production via TLR3 stimulation owing to the inhibition of IFN signaling. (A) In cells maintained at each temperature, we measured BAFF concentration 72 h after poly I:C stimulation. There was a significant difference in the BAFF production between the BEAS-2B cells grown at 37 °C and 33 °C, with low temperatures inhibiting BAFF production. (B) In cells maintained at each temperature, we measured IFN-β concentration 24 h after poly I:C stimulation. Low temperatures significantly enhanced IFN-β production. (C) BAFF production upon stimulation by IFN-β increased in a dose-dependent manner in cells grown at 37 °C. In contrast, in the low-temperature settings (33 °C), lower levels of BAFF were produced upon stimulation with IFN-β. The amount of BAFF produced was not affected by the IFN-β dose. (D) The expression of IFNAR1 (IFN receptor) was measured by SDS-PAGE and western blotting and was shown to decrease in the low-temperature settings (33 °C).

BAFF during viral infection, a major URTI, and airway epithelial cells could play important protective roles against viral infections. Indeed, McNamara et al. reported the importance of airway epithelial cells as a producer of BAFF [11]. Our study also showed that the stimulation of poly I:C with BEAS-2B cells produced IFN-β and downstream IFNAR, and that the JAK/signal transducer and activator of transcription (STAT) pathway played an important role in the production of BAFF in BEAS-2B cells. These results suggest that BAFF production in airway epithelial cells by poly I:C stimulation occurs by the self-stimulation of the IFN-β pathway, as demonstrated by Kato et al. [15].

Our study showed that the amount of BAFF produced by TLR3 stimulation significantly decreased in low-temperature settings. The results of the lactate dehydrogenase assay showed that low temperatures did not have cytotoxic effects on bronchial epithelial cells (data not shown). Moreover, the amount of IFN-β produced by TLR3 stimulation significantly increased in low-temperature settings. These results suggest that low temperature might inhibit IFN-β stimulation and/or the JAK-STAT pathway. Our study showed that the expression of type 1 IFN receptor decreased in BEAS-2B cells in low-temperature settings, which might be involved in the decline in IFN-β reactivity and decrease in BAFF production.

Wang et al. showed that cigarette smoke extracts inhibit the production of BAFF, and the amount of sIgA decreased in mice infected with influenza virus [16]. BAFF is involved in sIgA production, and these results implied that the amount of sIgA in the airway epithelium is mainly dependent on BAFF stimulation during viral infection.

We suggest that cold exposure can suppress BAFF production upon viral stimulation, and this might result in the inhibition of IgA secretion in airway epithelium, thereby increasing the risk of acquiring respiratory tract infections.

CRediT author statement

Yusuke Yoshino: Conceptualization, Methodology, Writing - Original Draft. Ai Yamamoto, Keita Misu, Yoshitaka Wakabayashi: Supervision. Takatoshi kitazawa: Writing - Review & Editing, Supervision. Yasuo Ota: Writing - Review & Editing, Supervision.

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Declaration of competing interest

The authors declare that they have no conflicts of interests.

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