Interleukin-1 β Mediates Virus-Induced M₂ Muscarinic Receptor Dysfunction and Airway Hyperreactivity

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Abstract

Respiratory viral infections are associated with the majority of asthma attacks. Inhibitory M₂ receptors on parasympathetic nerves, which normally limit acetylcholine (ACh) release, are dysfunctional after respiratory viral infection. Because IL-1 β is up-regulated during respiratory viral infections, we investigated whether IL-1 β mediates M₂ receptor dysfunction during parainfluenza virus infection. Virus-infected guinea pigs were pretreated with the IL-1 β antagonist anakinra. In the absence of anakinra, viral infection increased bronchoconstriction in response to vagal stimulation but not to intravenous ACh, and neuronal M₂ muscarinic receptors were dysfunctional. Pretreatment with anakinra prevented virus-induced increased bronchoconstriction and M₂ receptor dysfunction. Anakinra did not change smooth muscle M₃ muscarinic receptor response to ACh, lung viral loads, or blood and bronchoalveolar lavage leukocyte populations. Respiratory virus infection decreased M₂ receptor mRNA expression in parasympathetic ganglia extracted from infected animals, and this was prevented by blocking IL-1 β or TNF- α . Treatment of SK-N-SH neuroblastoma cells or primary cultures of guinea pig parasympathetic neurons with IL-1 β

directly decreased M₂ receptor mRNA, and this was not synergistic with TNF- α treatment. Treating guinea pig trachea segment with TNF- α or IL-1 β *in vitro* increased tracheal contractions in response to activation of airway nerves by electrical field stimulation. Blocking IL-1 β during TNF- α treatment prevented this hyperresponsiveness. These data show that virus-induced hyperreactivity and M₂ dysfunction involves IL-1 β and TNF- α , likely in sequence with TNF- α causing production of IL-1 β .

Keywords: asthma; IL-1 β ; TNF- α ; parainfluenza virus; parasympathetic nerves

Clinical Commentary

Human airway parasympathetic nerves express M₂ receptors, which limit acetylcholine release and are dysfunctional after virus infection. These data demonstrate that IL-1 β mediates virus-induced M₂ receptor dysfunction and mRNA down-regulation. Thus, these findings suggest new therapeutic approaches to treat virus-induced asthma attacks.

The majority of asthma attacks are associated with respiratory viral infection, which can be detected in 80% of acute asthma exacerbations in children (1) and in 50% of exacerbations in adults (2). Even in healthy individuals, respiratory viral infection can cause airway hyperreactivity and increase airway resistance (3–5), likely by disrupting the function of

parasympathetic nerves that provide autonomic control of the airways.

Parasympathetic nerves release acetylcholine (ACh) onto M₃ muscarinic receptors on airway smooth muscle, causing muscle contraction and bronchoconstriction (6–8). ACh also activates M₂ muscarinic receptors on postganglionic nerves, which inhibits

further ACh release and limits bronchoconstriction (9). Parainfluenza virus decreases M₂ muscarinic receptor function on parasympathetic nerves (10), and loss of M₂ receptor-mediated negative feedback increases ACh release onto airway smooth muscle (11). M₂ receptor dysfunction is found in some patients with asthma (12) and is associated with airway

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Figure 1. Parainfluenza virus antigen does not colocalize with airway nerves after infection in the lungs of guinea pigs. Guinea pig lungs infected with 1×10^6 TCID₅₀/ml were fixed, sectioned, and immunostained with a polyclonal antibody to parainfluenza virus antigen (red), a pan-neuronal nerve antibody to PGP9.5 (green), and 4',6-diamidino-2-phenylindole (DAPI) nuclear stain (blue). Viral antigen (red) is clearly seen in airway epithelial cells, and airway nerves (green) are clearly identified, but there is no colocalization of virus with nerves. Original magnification: $\times 40$ (bar, 50 μ m) ($n = 4$).

hyperreactivity after virus infection or double-stranded RNA production (13, 14).

Previous studies indicate that inflammatory cytokines are mechanisms for M_2 receptor dysfunction. *In vitro*, M_2 receptor expression is decreased on parasympathetic (15) and increased on sympathetic (16) cultured neurons by IFN- γ . In addition, TNF- α decreases M_2 receptor gene expression in neuronal cells (17). Blocking TNF- α with etanercept prevents airway hyperreactivity and M_2 receptor dysfunction during virus infection (18).

IL-1 β is a cytokine that is increased during virus infection and has been often implicated in asthma (19–21). Even in the absence of respiratory viral infection, IL-1 β is elevated in bronchoalveolar lavage (BAL) fluid and bronchial epithelium from both symptomatic and asymptomatic patients with asthma (19–22). Blocking IL-1 β prevents airway hyperreactivity in ozone-exposed and antigen-challenged animals (23, 24). Furthermore, parainfluenza virus-treated cells increase the production of IL-1 β (25, 26). However, the role of IL-1 β has not been investigated *in vivo* in a virus infection animal model.

To test the effects of IL-1 β on M_2 receptor function, we blocked IL-1 β with anakinra during parainfluenza virus infection in guinea pigs. We demonstrated that blocking IL-1 β prevented M_2 receptor dysfunction and that blocking either IL-1 β or TNF- α prevented a decrease in M_2 receptor expression after infection in parasympathetic nerves *in vivo*. We also showed that direct treatment of IL-1 β -induced down-regulation of M_2 receptor expression in cultured neurons. Furthermore, we showed that although either IL-1 β or TNF- α can increase airway contractile responses to nerve stimulation *in vitro*, TNF- α appears to do so through the production of IL-1 β .

Materials and Methods

Virus Infection and Treatments of Guinea Pigs

Parainfluenza virus was grown, purified, and titered as previously described (10). Dunkin-Hartley guinea pigs were intranasally infected as previously described (10). Some animals received the TNF- α blocker etanercept (Immunex, Thousand Oaks, CA) 24 hours before infection, and

some were treated with the IL-1 β blocker anakinra (Amgen, Thousand Oaks, CA) 30 minutes before infection and once daily thereafter. *In vivo* physiology experiments were done 4 days after infection. After experiments, the lungs were homogenized, RNA was extracted, and viral titers were assessed by real-time PCR.

Immunostaining

Lungs were fixed and stained for parainfluenza virus and the nerve-specific protein PGP9.5 as described in the online supplement.

In Vivo Measurement of Neuronal M_2 Receptor Function

Bronchoconstriction in response to vagal stimulation and intravenous ACh were measured in anesthetized, mechanically ventilated guinea pigs (see online supplement). M_2 receptor function was measured by the ability of the M_2 antagonist gallamine to potentiate the bronchoconstrictor response to vagal stimulation, as previously described (9).

BAL

At the end of each experiment, lungs were lavaged, and cell counts and differentials

Table 1. Baseline Physiology Measurements

	Weight (g)	Ppi (mm H ₂ O)	Heart Rate (bpm)	Systolic BP (mm Hg)	Diastolic BP (mm Hg)
Mock	401.6 $\pm$ 26.3	80 $\pm$ 9.5	235 $\pm$ 12.5	33.6 $\pm$ 3.2	13.6 $\pm$ 2.2
Virus	368.8 $\pm$ 21.5	76 $\pm$ 5.8	263 $\pm$ 10.9	40 $\pm$ 3.4	14.4 $\pm$ 1.4
Virus + vehicle	363.8 $\pm$ 2.0	99 $\pm$ 8.1	267 $\pm$ 21.7	35.6 $\pm$ 7.5	14.2 $\pm$ 2.1
Virus + anakinra	360.8 $\pm$ 14.0	97.5 $\pm$ 6.3	250 $\pm$ 4.1	35.5 $\pm$ 6.3	12.3 $\pm$ 0.6

Definitions of abbreviations: BP, blood pressure; bpm, beats per minute; Ppi, pulmonary inflation pressure. Data are represented as $\pm$ SEM.

were determined as described in the online supplement.

Blood

Blood leukocyte counts and differentials were determined as described in the online supplement.

Parasympathetic Ganglia Dissection

Guinea pigs tracheas were removed, opened anteriorly, pinned lumen side down, and stained for 30 minutes on ice with 0.05% neutral red solution (Fisher, Waltham, MA) made in RNase-free PBS. Tracheas were rinsed with cold RNase-free PBS, and the airway smooth muscle was separated from ganglia with sterile Dumont no. 5 forceps (Fine Science Tools, Foster City, CA) using a Nikon SMZ1000 dissecting stereomicroscope (Nikon, Tokyo, Japan). Each process of the ganglia was severed, and non-neuronal tissue was cleaned away. Ganglia from each trachea were pooled together and stored at -80°C for RNA isolation using the Cells-to-CT kit (Life Technologies, Grand Island, NY).

Tissue Culture

Human SK-N-SH neuroblastoma cells (ATCC) were grown in 6-well plates as described in the online supplement. When they reached 70 to 80% confluence, cells were either untreated or treated with 0.01 to 100 ng/ml human recombinant IL-1 β (Sigma, St. Louis, MO). Six hours later, RNA was isolated for measurement of M_2 receptor gene expression as described in the online supplement.

Quantitative RT-PCR

M_2 receptor and PGP9.5 RNA expression in SK-N-SH cells and parasympathetic ganglia were determined using RT-PCR as described in the online supplement.

Organ Bath Experiments

Guinea pig tracheas were cut transversely into ring segments consisting of three cartilage rings and placed in 96-well culture wells with Dulbecco's modified Eagle medium (Gibco, Carlsbad, CA) with penicillin and streptomycin. Segments were untreated or treated with guinea pig recombinant TNF- α (100 ng/ml), human recombinant IL-1 β (100 ng/ml), human recombinant IL-1RA (anakinra, 100 ng/ml), or etanercept (100 ng/ml) for 4 days. Some segments were treated with a combination

of TNF- α (100 ng/ml) and anakinra (100 ng/ml) or IL-1 β (100 ng/ml) and etanercept (100 ng/ml) for 4 days.

Trachea ring contractions were measured in a 5-ml organ bath (Radnoti Glass Technology, Monrovia, CA) as described in the online supplement.

IL-1 β ELISA

IL-1 β protein levels were determined as described in the online supplement.

Statistical Data Analysis

Data were analyzed as described in the online supplement.

Figure 2. Parainfluenza virus infection induces pulmonary M_2 receptor dysfunction, which is prevented by blocking IL-1 β . Guinea pigs were treated with anakinra (30 mg/kg, intraperitoneally), an IL-1 β receptor antagonist, 30 minutes before infection or PBS mock infection. Bronchoconstriction were induced by electrical stimulation of both of the vagus nerves (15 Hz, 10 V, 0.2-ms duration; 5 s on, 60 s off intervals). M_2 receptor function was measured as the potentiation in bronchoconstrictions after gallamine (0.1–10 mg/kg, intravenously), an M_2 receptor antagonist. (A) Gallamine potentiated bronchoconstrictions in controlled mock-infected animals. The parainfluenza virus-infected animals had a significantly reduced response to gallamine, indicating M_2 receptor dysfunction. Pretreatment with anakinra to block IL-1 β prevented M_2 receptor dysfunction in virus-infected animals. (B) Gallamine blocked cardiac M_2 receptor function and prevented the fall in heart rate, which was unaffected by parainfluenza virus infection or anakinra treatment. (C) Acetylcholine-induced (1–10 $\mu\text{g/kg}$, intravenously) bronchoconstrictions (C) and bradycardia (D) were not changed with virus infection or anakinra treatment. Data are represented as ratio of after gallamine to before gallamine in (A) and (B). Data are expressed as $\pm$ SEM ($n = 4$ –5). * $P < 0.05$ and ** $P < 0.005$ compared with the mock-infected control group. # $P < 0.05$ compared with infected group.

Results

Parainfluenza Virus Does Not Infect Airway Neurons *In Vivo*

Because previous studies indicate that respiratory syncytial virus and human metapneumovirus infect airway neurons (27, 28) and that parainfluenza virus infects olfactory bulb neurons (29), we first determined if parainfluenza virus infects airway neurons *in vivo*. Guinea pig airways were removed 4 days after infection and stained with antibodies to the pan-neuronal nerve marker PGP9.5 and parainfluenza virus antigen. Parainfluenza virus antigen localized to the airway epithelium. There was no colocalization of viral protein and nerves in the airways of infected animals (Figure 1). Thus, parainfluenza does not infect airway nerves *in vivo*, and the changes in neuronal control are likely due to mediators released during infection.

Effects of Blocking IL-1 β on Airway M₂ Muscarinic Receptor Function in Virus-Infected Guinea Pigs

Guinea pigs were matched by age before experiments. There were no differences in heart rate, blood pressure, or weights among the treatment groups of animals (Table 1).

Electrical stimulation of both vagus nerves (15 Hz, 10 V, 0.2-ms duration; 5 s on, 60 s off intervals) produced reproducible, frequency-dependent bronchoconstriction measured as an increase in pulmonary inflation pressure above baseline. M₂ receptor function was measured as the ability of gallamine (0.1–10 mg/kg, intravenously), an M₂ receptor antagonist, to potentiate vagally induced bronchoconstriction. This increase is due to blockade of inhibitory M₂ neuronal receptors, increasing ACh release. Parainfluenza virus-infected animals had a decreased response to gallamine compared with mock-infected animals, indicating M₂ receptor dysfunction (Figure 2A). Animals pretreated with anakinra to block IL-1 β had normal M₂ receptor function after infection.

Effects of Respiratory Virus Infection and of Blocking IL-1 β on Airway Smooth Muscle and Cardiac Muscle

ACh (1–10 μ g/kg, intravenously) produced dose-dependent bronchoconstriction by stimulating M₃ muscarinic receptors on

airway smooth muscle (Figure 2C) and dose-dependent bradycardia by stimulating M₂ muscarinic receptors in the heart (Figure 2D). Neither virus nor anakinra affected ACh-induced bronchoconstriction or bradycardia compared with controls, indicating that cardiac and airway smooth muscle responsiveness was not altered by infection or drug treatment. Gallamine (0.1–10 mg/kg, intravenously) blocked M₂ receptors in the heart and prevented vagally induced bradycardia similarly in all groups (Figure 2B).

Effect of Respiratory Virus Infection and of Blocking IL-1 β on Inflammatory Cells

Parainfluenza virus infection decreased total inflammatory cells in the blood (Figure 3A). The leukopenia was largely due to decreased neutrophils and lymphocytes, and was unaffected by anakinra. In the BAL, virus infection increased total inflammatory cells, predominantly macrophages and neutrophils, but this was also not changed by anakinra (Figure 3B). The changes in cell populations in the blood and BAL were not significantly

different between virus-infected and anakinra-treated animals.

Effects of Respiratory Virus Infection and Blocking IL-1 β and TNF- α on M₂ Receptor Expression in Parasympathetic Nerves *In Vivo*

Parasympathetic ganglia were dissected from tracheas of virus-infected guinea pigs to measure M₂ receptor expression (Figure 4A). Parainfluenza virus infection caused a $66.92 \pm 10.51\%$ decrease in M₂ receptor gene expression compared with mock-infected animals (Figure 4B).

Virus-infected animals treated with etanercept or anakinra had normal M₂ receptor expression. PGP9.5 mRNA expression was also assessed to confirm neuronal tissue (data not shown).

Effects of Blocking IL-1 β or TNF- α on Viral Replication in the Lungs

Virus-infected animals had an average titer of $1.9 \pm 0.6 \times 10^4$ TCID₅₀ equivalents/mg, which was similar to virus-infected animals treated with anakinra ($6.43 \pm 5.2 \times 10^3$ TCID₅₀ equivalents/mg) or etanercept ($3.71 \pm 1.5 \times 10^4$ TCID₅₀ equivalents/mg) (Figure 5).

Figure 3. Parainfluenza infection alters leukocytes in the blood and bronchoalveolar lavage (BAL). Neither is unaffected by anakinra treatment. (A) Infection decreased total blood leukocytes, mainly by decreasing neutrophils and lymphocytes. This was not significantly different with anakinra treatment ($n = 8-9$). (B) Infection increased total BAL leukocytes, mainly by increasing neutrophils and macrophages. This was not significantly different with anakinra treatment ($n = 7-9$). Data are represented as $\pm$ SEM. * $P < 0.05$ significance compared with control group. ** $P < 0.01$ significance compared with control group.

Figure 4. *In vivo* parainfluenza virus infection decreases M₂ receptor mRNA expression in airway parasympathetic ganglia. This decrease is prevented by treating with either anakinra (30 mg/kg, intraperitoneally, daily) or etanercept (3 mg/kg, intraperitoneally, as a single dose). (A) Serial images demonstrating the dissection of one parasympathetic ganglion from the tracheal smooth muscle stained with neutral red. (B) M₂ receptor mRNA expression in airway parasympathetic ganglia. Data are represented as $\pm$ SEM ($n = 5-8$). * $P < 0.05$ compared with the mock-infected control group.

Effect of IL-1 β and TNF- α on M₂ Receptor Expression in Neurons *In Vitro*

IL-1 β decreased M₂ receptor mRNA in a dose-dependent fashion in human SK-N-SH neuroblastoma cells (Figure 6A). IL-1 β decreased M₂ mRNA by $92.61 \pm 0.86\%$ at 100 ng/ml and had an approximate IC₅₀ of 0.0138 ng/ml. To test whether IL-1 β and TNF- α were synergistic, primary cultures of guinea pig airway parasympathetic neurons were treated with a combined dose of IL-1 β (0.001–0.01 ng/ml) and TNF- α (0.01–0.1 ng/ml) for 6 hours. Although both cytokines alone decreased M₂ receptor expression, there was no synergistic decrease when the cytokines were combined (Figure 6B).

Effects of TNF- α and IL-1 β Treatment on Electrical Field Stimulation- and MCh-Induced Contractions of Isolated Guinea Pig Tracheas

Tracheal segments gave reproducible contractions in response to electrical field

stimulation (EFS) (40 V, 1–30 Hz, 0.5-ms duration; 60 s on, 60 s off intervals). Contractions were blocked by tetrodotoxin (1 μ M), a voltage-gated sodium channel blocker, indicating that they were mediated by neurotransmitter release from airway nerves. Tracheal segments cultured with TNF- α (100 ng/ml) or IL-1 β (100 ng/ml) had increased contractile responses to EFS compared with untreated tissue (Figures 7A and 7B). Blocking IL-1 β (with anakinra) prevented TNF- α -mediated potentiation of EFS contractions (Figure 7A). However, blocking TNF- α (with etanercept) had no effect on IL-1 β -induced potentiation of EFS contractions (Figure 7B). These data suggest that TNF- α mediates increased contractile responses through the production of IL-1 β .

Tracheal segments gave reproducible contractions in response to MCh (10 μ M) or KCl (100 mM), both of which bypass nerves and induce contraction via M₃ receptors (MCh) or by depolarizing

smooth muscle (KCl) (data not shown). Neither IL-1 β nor TNF- α had any effect on contractions induced by MCh or KCl (Figure 7C), indicating that neither cytokine increased smooth muscle contractility.

IL- β production from supernatants of the cultured trachea rings was confirmed by ELISA (Figure 7D). IL-1 β was increased 5.91 ± 0.7 -fold when treated with TNF- α compared with untreated controls. IL-1 β could be measured even in the presence of anakinra. Trachea segments treated with TNF- α and anakinra also had a significant increase above controls (5.18 ± 0.31 -fold) at 24 hours. Anakinra alone had no effect on IL-1 β levels.

Discussion

We initially tested whether parainfluenza virus directly infects airway neurons because parasympathetic nerves mediate bronchoconstriction during asthma exacerbation (6, 10). Although the primary target of respiratory viruses is the epithelial cell, respiratory syncytial virus and human metapneumovirus infect airway neurons *in vivo* (27, 28). In addition, parainfluenza virus infects parasympathetic neurons *in vitro* (10, 15) and olfactory neurons *in vivo* (29). However, we demonstrated that parainfluenza virus did not infect airway nerves *in vivo* (Figure 1), suggesting that inflammatory mediators released during

Figure 5. Treatment of parainfluenza virus-infected animals with anakinra or etanercept did not change lung viral titers. Viral titers were normalized to 18S rRNA, and tissue culture infective dose (TCID₅₀) was determined from a parainfluenza standard curve. Data are shown as $\pm$ SEM ($n = 6-9$).

Figure 6. IL-1 β and TNF- α decrease M₂ receptor mRNA in nerve cells. (A) Treatment of human SK-N-SH neuroblastoma cells with IL-1 β (0.01–100 ng/ml) for 6 hours decreases M₂ receptor mRNA expression in a dose-dependent fashion. (B) Treatment of primary cultures of guinea pig airway parasympathetic neurons with combined doses of IL-1 β (0.001–0.01 ng/ml) and TNF- α (0.01–0.1 ng/ml) for 6 hours decreases M₂ receptor mRNA expression, but the effect of IL-1 β is not synergistic with TNF- α . Data are represented as $\pm$ SEM ($n = 3$). ** $P < 0.05$ significantly different from control group. *** $P < 0.0001$ significance compared with control group.

infection might be responsible for airway nerve dysfunction.

Respiratory virus infections change the neural control of the airways and are associated with the majority of asthma exacerbations (1, 2). We previously showed that blocking TNF- α with etanercept prevents virus-induced airway hyper-reactivity and M₂ receptor dysfunction (18). Here we show that virus-induced neuronal M₂ receptor dysfunction is also prevented by blocking IL-1 β with anakinra (Figure 2). The IL-1 β antagonist did not affect lung viral titers or white cell populations in the blood or BAL (Figures 3 and 5). Depleting macrophages (30) and granulocytes (13) has been shown to prevent M₂ receptor dysfunction during respiratory viral infection. However, the leukocytes in the BAL of anakinra-treated virus infected animals were not significantly different from the virus-infected alone groups.

IL-1 β decreased M₂ receptor mRNA expression in cultured neurons (Figure 6). Additionally, treatment of tracheal tissue with TNF- α *in vitro* increased the contractile response to EFS of airway nerves, and this effect was accompanied by increased production of IL-1 β and was prevented when IL-1 β was blocked with anakinra (Figure 7).

Treating tissues with IL-1 β also increased the contractile response to EFS of airway nerves, but this was not prevented by the TNF- α blocker etanercept (Figure 7). Our data strongly suggest that viral infection of the airways leads to production of TNF- α , which induces production of IL-1 β , and that IL-1 β mediates the loss of neuronal expression and function of M₂ receptors, causing airway hyper-reactivity.

Parainfluenza virus is known to decrease M₂ receptor expression in cultured parasympathetic neurons (10, 15). Here we show that this also occurs *in vivo*. Parainfluenza decreased neuronal M₂ receptor mRNA expression in ganglia from infected guinea pigs, and this was prevented by treating guinea pigs with either etanercept or anakinra, demonstrating a role *in vivo* for IL-1 β or TNF- α (Figure 4). This is the first demonstration that airway parasympathetic ganglia can be dissected from guinea pig tracheas and used for RNA extraction and measurement of gene expression.

We also demonstrated that TNF- α and IL-1 β did not work synergistically in the down-regulation of M₂ receptor mRNA in neurons. Our findings are different from those in human lung embryonic lung fibroblasts, where M₂ receptor expression

decreases only with combined IL-1 β and TNF- α treatment (31). We showed that either TNF- α or IL-1 β alone decreased M₂ receptor expression in human SK-N-SH neuroblastoma cells and in primary cultures of guinea pig airway parasympathetic neurons (Figures 6A and 6B). This difference between the regulation of M₂ receptor expression in fibroblasts and its regulation in neurons, and in particular airway parasympathetic neurons, demonstrates the importance of carrying out *in vitro* studies in cells relevant to the *in vivo* finding.

M₂ receptor dysfunction is found in some patients with asthma (12), whereas other patients have normal M₂ receptors that only become dysfunctional during virus infection (32). There is a large population of patients with severe refractory asthma who are difficult to treat and are in need of novel therapies (33–35). Given that 80% of asthma exacerbations in children and 50% in adults are attributed to respiratory virus infections (1, 2) and that there are no therapies available for the common respiratory viruses detected during acute asthma attacks (1), this provides an opportunity for new therapies to treat virus-induced airway hyperreactivity. Anakinra, or other treatments to block IL-1 β , may be helpful in patients with severe asthma on inhaled corticosteroid who are still symptomatic (36, 37). Furthermore, some patients with severe asthma who are resistant to traditional treatments have benefited from anti-TNF- α treatment (38–40). These patients may have further relief with an IL-1 receptor antagonist treatment either alone or with etanercept.

In conclusion, we have shown that virus-induced airway hyperreactivity and loss of M₂ receptor function are the result of IL-1 β -mediated loss of M₂ receptor gene expression in parasympathetic neurons. Our studies suggest that virus-induced TNF- α expression stimulates IL-1 β production, explaining why blocking either TNF- α or IL-1 β prevents M₂ receptor dysfunction. Thus, therapeutic strategies targeting TNF- α , IL-1 β , or both may be effective in preventing virus-induced asthma attacks. ■

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Figure 7. IL-1 β (100 ng/ml) and TNF- α (100 ng/ml) increase guinea pig tracheal contractions in response to electrical field stimulation of airway nerves *in vitro* (40 V, 1–30 Hz, 0.5-ms duration; 60 s on, 60 s off intervals). Blocking IL-1 β with anakinra (IL-1RA, 100 ng/ml) prevents the effect of TNF- α (A), but blocking TNF- α with etanercept (100 ng/ml) does not prevent the effect of IL-1 β (B). (C) MCh (10 μ M)-induced contractions were not different between treatment groups. (D) IL-1 β was produced in airway segments treated with TNF- α alone or with TNF- α and IL-1RA. Data are represented as $\pm$ SEM ($n = 4$ –9). * $P < 0.05$, significantly different from control group. ** $P < 0.01$ and *** $P < 0.001$, significantly different from control group.

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