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# Gamma-interferon modifies guinea pig airway functions *in vitro*

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*Gamma-interferon modifies guinea pig airway functions* in vitro. *H. Chen, M. Munakata, M. Amishima, H. Ukita, Y. Masaki, Y. Homma, Y. Kawakami. ©ERS Journals Ltd 1994.*
**ABSTRACT: Cytokines produced by T-lymphocytes may have significant roles in the airway inflammation seen in bronchial asthma. Gamma interferon (IFN-γ), a T-cell derived cytokine, is known to modify functions of both immune and non-immune cells. In this study, we investigated whether IFN-γ can modify guinea pig airway functions *in vitro*.**

**The isometric tension of guinea pig airway strips was measured in a tissue bath filled with Krebs-Henseleit solution. Contracting responses to carbachol and KCl, and the relaxing response to isoproterenol (ISO) were examined. Effects of IFN-γ were examined by comparing responses of the strips incubated with or without IFN-γ (1000 U·ml$^{-1}$ ; 25,000 U·ml$^{-1}$).**

**Contracting responses to carbachol and KCl were not affected by the incubation with IFN-γ other than slight increased in maximum contraction by carbachol after 5 hours incubation with 25,000 U·ml$^{-1}$ of IFN-γ. Both 1 and 5 h incubation of strips with 25,000 U·ml$^{-1}$ IFN-γ significantly increased the sensitivity to ISO ($p<0.01$ and $p<0.05$, respectively) without affecting maximum relaxation. The effect of IFN-γ on ISO relaxation was abolished by the denudation of airway epithelium from strips, indomethacin (2 μM), and cycloheximide (70 μM) but not by N$^{\omega}$-nitro-L-arginine methyl ester (30 μM). In addition, heat-inactivated INF-γ and bacterial endotoxin (LPS, 0.625 pg·ml$^{-1}$) had no effect on ISO relaxation.**

**These results suggest that IFN-γ is able to modify airway smooth muscle response to β-adrenergic agonist by inducing release of prostanoids from airway epithelium.**
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Bronchial asthma has been recognized as an airway inflammatory process in which several immune cells such as neutrophils, eosinophils, lymphocytes, and macrophages, may interact to generate complex pathological and pathophysiological abnormalities [1, 2]. In this process, the role of the T-lymphocytes in the regulation and expression of the inflammation has received a fair amount of attention. An increased number of lymphocytes was a consistent finding in asthmatic airway tissue taken both by autopsy and biopsy [3, 4]. Significantly higher numbers of IL-2 receptor-positive T-cells [5] and increased natural killer cell activity seen in the peripheral blood of asthmatic patients [6] suggested the important roles played by activated T-cells. In addition, T-cell derived lymphokines are intimately involved in the regulation of IgE production [7]. These data suggest that lymphokines may play an important role in the development of asthma and airway hyperresponsiveness [8].

Gamma interferon (IFN-γ), a T-cell derived cytokine, was discovered in 1957 as an anti-viral protein produced by virus-infected cells [9]. Since viral respiratory infections are well known to increase bronchial responsiveness in normal and asthmatic subjects [10, 11], there has been a great deal of interest centring on IFN-γ in inflammatory responses with respect to asthmatic patients [12–14]. These studies were mainly focused on the effects of IFN-γ on immune cells. However, IFN-γ also exhibits a variety of biological activity in non-immune cells. In the case of other cytokines, such as interleukin 1, direct action on vascular smooth muscle has been recognized [15, 16]. This information raises the possibility that direct action of IFN-γ on airway smooth muscle might play some role in asthma and airway hyperreactivity.

The aim of this study is to investigate this possible action of IFN-γ on airway smooth muscle. Airway smooth muscle functions can be altered by the modification of both contracting and relaxing responses. In addition, both the stimulation of specific receptors by agonists and the changes in membrane potentials of smooth muscle cells can induce contraction and relaxation of smooth muscle. Since IFN-γ might affect only some of these pathways, we examined the effects of IFN-γ on two contractile agents, muscarinic receptor agonist (carbachol) and a membrane depolarizer (KCl), and on a relaxing agent, the β-adrenergic receptor agonist (isoproterenol). In addition, since there is a

possibility that the action of IFN-γ is not directly on smooth muscle but is mediated through its action on surrounding tissue, we also examined whether denudation of epithelium from airway strips can alter the effect of IFN-γ.

## Materials and methods

### *Measurements*

Male guinea pigs, weighing 300–400 g, were used for the experiment and killed by a blow to the head. The tracheas were removed, dissected free of extraneous tissue and cut into rings containing two cartilages. A tracheal strip was prepared by cutting open the ring at the cartilage opposite the smooth muscle. This technique preserves the epithelium lining. In some strips, the epithelium was removed by gently rubbing the luminal surface with a cotton swab. The lower end of the open tracheal strip was attached to a metal hook by a loop of silk thread at the base of an organ bath filled with 5 ml Krebs-Henseleit (K-H) solution (37°C) of the following composition (mM): NaCl 113.08; KCl 5.15; $NaH_2PO_4$ 0.97; glucose 10.67; $MgSO_4$ 0.66; $CaCl_2$ 2.42; and $NaHCO_3$ 24.04. K-H solution was continuously gassed with a 5% $CO_2$ and 95% $O_2$ mixture. The upper end was attached in the same manner to an isometric transducer (NIHON KODEN, TB-652T) by which the force of contraction produced by the strips were measured and the concentration-response curves were displayed on a six-channel pen recorder (RIKADENKI). All initial loads were set at approximately 2 g before the initiation of studies and 2 g of load was maintained for about 1 h for each strip until the drugs were given when a steady state had been reached.

### *Drugs and chemicals*

Recombinant human IFN-γ was kindly supplied in sterile vials by the Shionogi Seiyaku Company. Each vial contained $5\times10^6$ units (U) (250 μg) of IFN-γ with less than 125 pg of bacterial endotoxin. K-H solution was used to dilute it down to $5\times10^5$ U·ml$^{-1}$ and it was stored at 80°C till use. The dosage of IFN-γ used in the bath was 1,000 U·ml$^{-1}$ (50 ng·ml$^{-1}$) or 25,000 U·ml$^{-1}$ (1,250 ng·ml$^{-1}$), which contains less than 0.025 pg·ml$^{-1}$ and 0.625 pg·ml$^{-1}$ of bacterial endotoxin, respectively. K-H solution was received as a vehicle in control groups with equivalent volumes. Fresh drug solutions such as carbachol, isoproterenol (ISO), papaverine and KCl, were made up on the day of the experiment and diluted with K-H solution or distilled water. All drugs were obtained from Sigma. All concentrations refer to the final bath concentration.

### *Experimental protocol*

In experiments, the strips of a guinea pig trachea in the baths with K-H solution were washed for 10 s at 10 min intervals × 5 times. The concentration-response curves relating to airway smooth muscle contraction for carbachol and KCl concentrations, ranging from 3 nM to 10.0 μM and from 10 mM to 100 mM, respectively, were constructed by cumulatively increasing concentrations of contractile agents, which were increased only when the contraction in response to the previous concentration had stabilized. The strips were washed again as described above, and then IFN-γ or K-H solution as a vehicle was put into each 5 ml bath. Incubation periods were set for one and five hours. Concentration-response curves with equivalent concentrations of contractile agents were likewise obtained in the presence or absence of IFN-γ. With two contractile agents, such as carbachol and KCl, concentration-response studies in the control group were performed with each strip to confirm repeat repeatability.

To examine the relaxing response curves for ISO, the concentration of carbachol required to produce half maximal contraction ($EC_{50}$) was first determined for each strip as described above. Then the tissue was washed five times with fresh K-H solution. After adjusting initial load to 2 g, strips were incubated with 1000 U·ml$^{-1}$ (50 ng·ml$^{-1}$) or 25,000 U·ml$^{-1}$ (1250 ng·ml$^{-1}$) of IFN-γ for one or five hours. At the end of the incubation period, the strip was contracted with its $EC_{50}$ of carbachol. On obtaining a stable contraction, ISO was introduced into each bath with increased concentrations ranging from 1 nM to 10 μM. Papaverine (200 μM), which was confirmed to produce complete relaxation of guinea pig tracheal strips, was added at the end of the experiment to evaluate whether maximum relaxation was achieved with the highest concentration of ISO. For the control, K-H solution was added as a vehicle instead of IFN-γ and the same protocol was applied. Since it is known that there is a tachyphylaxis for the ISO response, the protocol, in which the ISO response was repeated twice to compare the responses before and after incubation of strips with IFN-γ was abandoned.

To examine the specificity of IFN-γ, following three experiments were done. First, to examine whether human recombinant IFN-γ can act on guinea pig cells, guinea pig peritoneal lavage cells (about 60% of the cells were macrophages) were collected and incubated with and without 1000 U·ml$^{-1}$ of IFN-γ for 72 hours. After the incubation, cells were spun down and stained with monoclonal antibody to guinea pig major histocompatibility comples (MHC) class II antigen (MSgp 8, SEROTEC). In order to eliminate nonspecific action of IFN-γ, effect of heat-inactivated IFN-γ (25,000 U·ml$^{-1}$ heated at 70° C for 1 h) on ISO relaxation was examined. In addition, since it is known that recombinant human IFN-γ is contaminated with trace amount of bacterial endotoxin, effect of bacterial endotoxin (lipopolysaccharide; LPS) was also examined. For the IFN-γ we used in this study, the amount of bacterial endotoxin contaminated is less than 0.625 pg·ml$^{-1}$. For this reason, we examined the effect of LPS (0.625 pg·ml$^{-1}$) on ISO relaxation.

In another series of experiments, to understand the mechanism of action of IFN-γ on ISO relaxation, the effect of airway epithelial denudation from the strips was examined. In addition, the effects of the following

drugs were examined. First, in order to establish whether protein synthesis is required for the effects of IFN-γ, the effect of cycloheximide (70 μM) was examined; to investigate the mechanism of the effects of IFN-γ, indomethacin (a cyclooxygenase inhibitor, 2 μM) and $N^{\omega}$-nitro-L-arginine methyl ester ($N^{\omega}$-NAME, an inhibitor of nitric oxide synthesis, 30 μM) were also examined. Controls in this study means vehicle control.

*Data analysis*

To eliminate possible damage to the smooth muscle during preparation of the strips, strips were discarded when they did not produce the force of contraction more than 1.0 g with the highest concentration of carbachol. For KCl experiments, 10 μM carbachol was added to the baths at the end of the second KCl dose-response curve recorded to examine whether the maximal contractile force was more than 1.0 g. We determined maximum responses in grams and the concentrations of two contracting agents and a relaxing agent required to produce half-maximal contraction ($EC_{50}$) and relaxation ($IC_{50}$). For ISO, the maximum response was expressed as a percentage of complete relaxation induced with papaverine. The individual determinations of $EC_{50}$ and $IC_{50}$ for carbachol and ISO, respectively, were then converted to log values from which the arithmetic means of the log values were calculated.

The maximal tensions and $EC_{50}$s for carbachol and KCl were compared both before and after the incubation with IFN-γ and between 1,000 U·ml$^{-1}$ or 25,000 U·ml$^{-1}$ IFN-γ-treated groups and the control for the same incubation period. In addition, changes in both maximum tension and $EC_{50}$ before and after incubation with IFN-γ or vehicle were calculated and compared. For ISO responses, maximum relaxation and $IC_{50}$ were compared between 1,000 U·ml$^{-1}$ or 25,000 U·ml$^{-1}$ IFN-γ-treated groups and the control.

*Statistics*

All values are expressed as mean±SEM. Student's paired t-test with two sided hypothesis was used for the comparison of the data before and after incubation with agents. Student's unpaired t-test with two sided hypothesis was used for the effect of inhibitors. Comparisons among groups with or without IFN-γ were performed by a one way analysis of variance (ANOVA). A p value $<0.05$ was considered significant.

## Results

*Effects of IFN-γ on carbachol contraction*

The mean maximal contractions and the changes before and after the incubation with IFN-γ or vehicle are shown in table 1. One hour incubation period did not alter the maximum response, however, five hour incubation period significantly reduced maximum contraction in control strips. This reduction was also found in IFN-γ treated strips. When the difference in maximum contractions before and after five hours incubation was compared, the reduction of maximum contraction in 25,000 U·ml$^{-1}$ IFN-γ treated strips was smaller than in control and in 1,000 U·ml$^{-1}$ IFN-γ treated strip ($p<0.05$).

Table 1. – Effects of IFN-γ on carbachol maximum contraction

| | Before g | After g | Δ g | (n) |
|---|---|---|---|---|
| 1 hr Incubation with IFN-γ | | | | |
| Control | 2.07±0.31 | 2.14±0.31 | 0.07±0.10 | (8) |
| IFN-γ (1,000 U·ml$^{-1}$) | 1.60±0.15 | 1.67±0.18 | 0.07±0.13 | (9) |
| IFN-γ (25,000 U·ml$^{-1}$) | 1.35±0.12 | 1.42±0.15 | 0.07±0.06 | (7) |
| 5 hr Incubation with IFN-γ | | | | |
| Control | 2.01±0.17 | 1.49±0.17* | -0.53±0.06 | (8) |
| IFN-γ (1,000 U·ml$^{-1}$) | 1.90±0.15 | 1.37±0.14* | -0.53±0.03 | (8) |
| IFN-γ (25,000 U·ml$^{-1}$) | 1.66±0.15 | 1.25±0.15* | -0.35±0.05† | (8) |

IFN-γ: gamma interferon; Δ: difference (after-before) in maximum contraction; *: significantly different from "before" incubation $p<0.05$. †: Significantly different from control and IFN-γ

The log($EC_{50}$)s and their changes before and after the incubation with IFN-γ or vehicle are shown on table 2. After one hour incubation, log($EC_{50}$) value was significantly increased in 25,000 U·ml$^{-1}$ IFN-γ treated strips. For five hours incubation, statistically significant increase in log($EC_{50}$) value was seen in all three groups. When the changes in log($EC_{50}$) value before and after the incubation were compared, no statistically significant difference was detected among three groups both for one and five hours incubation.

*Effect of IFN-γ on KCl contraction*

The mean maximal contractions and the changes before and after the incubation with IFN-γ or vehicle are shown in table 3. One hour incubation did not alter the maximum responses and $EC_{50}$ values except for the maximum response in control tissue, in which there was a slight but significant increase in maximum response after the incubation ($p<0.05$). There was no significant difference in maximum responses after five hours incubation period. When the difference in maximum contraction was compared, no statistically significant changes were detected among three groups both for one and five hours incubation.

Table 2. – Effects of IFN-γ on carbachol log($EC_{50}$)

| | Before | After | Δ | (n) |
|---|---|---|---|---|
| 1 hr Incubation with IFN-γ | | | | |
| Control | -7.19±0.02 | -7.07±0.22 | 0.11±0.07 | (8) |
| IFN-γ (1,000 U·ml$^{-1}$) | -6.93±0.10 | -6.72±0.06 | 0.21±0.11 | (9) |
| IFN-γ (25,000 U·ml$^{-1}$) | -7.12±0.14 | -6.93±0.17* | 0.20±0.06 | (7) |
| 5 hr Incubation with IFN-γ | | | | |
| Control | -7.06±0.04 | -6.76±0.09* | 0.32±0.11 | (8) |
| IFN-γ (1,000 U·ml$^{-1}$) | -7.10±0.04 | -6.68±0.06* | 0.41±0.09 | (8) |
| IFN-γ (25,000 U·ml$^{-1}$) | -7.11±0.04 | -6.92±0.06* | 0.39±0.04 | (8) |

$EC_{50}$: concentration required to produce half-maximal contraction. IFN-γ: gamma interferon; Δ: difference (after-before) in log($EC_{50}$). n: number of experiments.

Table 3. – Effects of IFN-γ on KCl maximum contraction

| | Before g | After g | Δ g | (n) |
|---|---|---|---|---|
| 1 hr Incubation with IFN-γ | | | | |
| Control | 1.10±0.13 | 1.23±0.13* | 0.13±0.04 | (6) |
| IFN-γ (1,000 U·ml$^{-1}$) | 0.80±0.05 | 0.91±0.05 | 0.10±0.05 | (5) |
| IFN-γ (25,000 U·ml$^{-1}$) | 0.93±0.07 | 1.00±0.05 | 0.07±0.05 | (6) |
| 5 hr Incubation with IFN-γ | | | | |
| Control | 1.25±0.10 | 1.18±0.13 | -0.07±0.09 | (8) |
| IFN-γ (1,000 U·ml$^{-1}$) | 1.16±0.12 | 1.20±0.14 | 0.04±0.11 | (5) |
| IFN-γ (25,000 U·ml$^{-1}$) | 1.07±0.05 | 1.06±0.07 | -0.02±0.05 | (8) |

IFN-γ: gamma interferon; Δ: difference (after-before) in maximum contraction. *: significantly different from "before" incubation, p<0.05. n: number of experiments.

The $EC_{50}$s and their changes before and after the incubation with IFN-γ or vehicle are shown in table 4. For one hour incubation, there was no significant difference in $EC_{50}$ for KCl was detected. For five hour incubation, increase in $EC_{50}$ after the incubation was seen in all three groups (p<0.05 in control and 1,000 U·ml$^{-1}$ IFN-γ treated groups and p<0.01 in 25,000 U·ml$^{-1}$ IFN-γ treated group). When the changes in $EC_{50}$ before and after the incubation with and without IFN-γ (1,000 U·ml$^{-1}$ or 25,000 U·ml$^{-1}$) of IFN-γ were compared, there was no difference among three groups.

*Effect of IFN-γ on Isoproterenol relaxation*

Results with ISO in epithelium intact preparation are shown in table 5 and figure 1. ISO caused complete relaxation of both IFN-γ-treated and untreated preparations, and there was no difference in maximum responses. In contrast, after one hour incubation, the mean±SEM of log($IC_{50}$) values in the control and in 25,000 U·ml$^{-1}$ IFN-γ group were -7.58±0.07 and -7.96±0.07, respectively. A significant difference between them was found (p<0.01). The log($IC_{50}$) values for five hours incubation period were -7.99±0.14 in the control and -8.42±0.13 in the 25,000 U·ml$^{-1}$ IFN-γ group; there was also a significant difference noted between them (p<0.05). Log($IC_{50}$) values for the tissue incubated with 1,000 U·ml$^{-1}$ IFN-γ for one or five hours fell between the control and the 25,000 U·ml$^{-1}$ group.

Table 4. – Effects of IFN-γ on KCl $EC_{50}$

| | Before | After | Δ | (n) |
|---|---|---|---|---|
| 1 hr Incubation with IFN-γ | | | | |
| Control | 26.0±2.16 | 28.5±1.73 | 2.50±1.87 | (6) |
| IFN-γ (1,000 U·ml$^{-1}$) | 23.1±1.32 | 24.4±0.51 | 1.36±1.36 | (5) |
| IFN-γ (25,000 U·ml$^{-1}$) | 24.8±1.27 | 26.3±1.17 | 1.53±1.02 | (6) |
| 5 hr Incubation with IFN-γ | | | | |
| Control | 24.3±1.25 | 27.8±0.40* | 3.56±1.12 | (8) |
| IFN-γ (1,000 U·ml$^{-1}$) | 26.3±2.96 | 31.3±1.85* | 5.00±1.15 | (5) |
| IFN-γ (25,000 U·ml$^{-1}$) | 26.5±0.99 | 31.9±1.81** | 5.40±1.30 | (8) |

IFN-γ: gamma interferon; $EC_{50}$: concentration required to produce half-maximal contraction. Δ: difference (after-before) in $EC_{50}$; *, **: significantly different from "before" incubation, p<0.05 and <0.01, respectively. n: number of experiments.

Table 5. – Effects of IFN-γ in isoproterenol relaxation (with epithelium)

| | Maximum relaxation (%) | Log($IC_{50}$) | (n) |
|---|---|---|---|
| 1 hr Incubation with IFN-γ | | | |
| Control | 100 | -7.58±0.07 | (8) |
| IFN-γ (1,000 U·ml$^{-1}$) | 100 | -7.70±0.08 | (8) |
| IFN-γ (25,000 U·ml$^{-1}$) | 100 | -7.96±0.07** | (8) |
| 5 hr Incubation with IFN-γ | | | |
| Control | 100 | -7.99±0.14 | (8) |
| IFN-γ (1,000 U·ml$^{-1}$) | 100 | -8.06±0.06 | (8) |
| IFN-γ (25,000 U·ml$^{-1}$) | 100 | -8.42±0.13* | (7) |

IFN-γ: gamma interferon; $IC_{50}$: concentration required to produce half-maximal relaxation. *, **: significantly different from control, p<0.05 and <0.01, respectively. n: number of experiments.

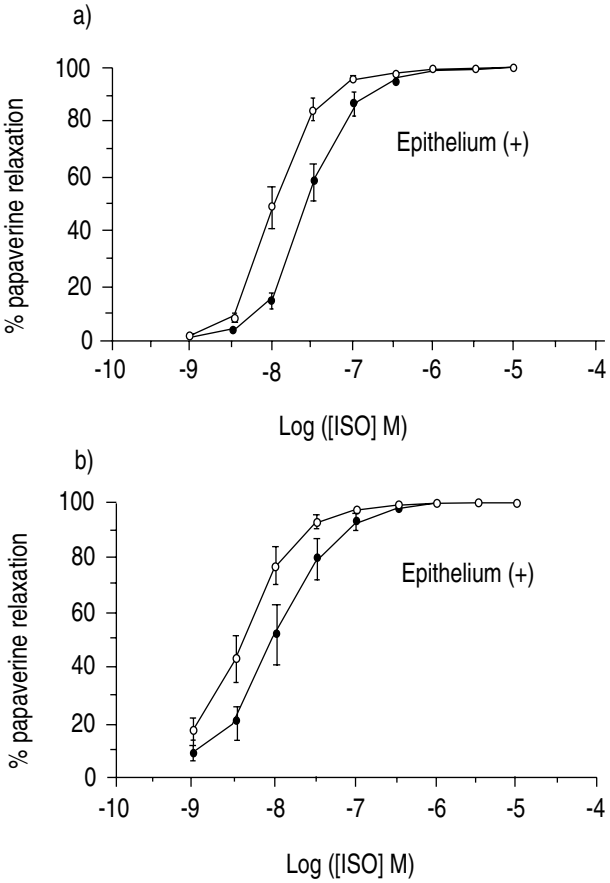


Fig. 1. – Concentration-response curves, showing arithmetic means with SEM, for isoproterenol (ISO) from tracheal strips with intact epithelium in the presence of 25,000 U·ml$^{-1}$ gamma interferon (IFN-γ) (○) and in control (●) for a) 1 hour and b) 5 hours. Response expressed as a percentage of maximal relaxation induced with 200 μM papaverine. There is a significant leftward shift in concentration response curves of strips incubated with IFN-γ from control line (p<0.01).

Results with ISO in epithelium denuded preparation are shown in table 6 and figure 2. ISO also caused complete relaxation of both IFN-γ-treated and untreated preparations, and there was no difference in maximum responses. For log($IC_{50}$) values, no difference was found between the tissue incubated with or without IFN-γ both for one and five hours incubation.

Table 6. – Effects of IFN-γ on isoproterenol relaxation (without epithelium)

| | Maximum relaxation (%) | Log($IC_{50}$) | (n) |
|---|---|---|---|
| 1 hr Incubation with IFN-γ | | | |
| Control | 100 | -8.16±0.05 | (7) |
| IFN-γ (1,000 U·ml$^{-1}$) | 100 | -8.15±0.04 | (7) |
| IFN-γ (25,000 U·ml$^{-1}$) | 100 | -8.13±0.07 | (7) |
| 5 hr Incubation with IFN-γ | | | |
| Control | 100 | -8.41±0.05 | (8) |
| IFN-γ (1,000 U·ml$^{-1}$) | 100 | -8.42±0.05 | (6) |
| IFN-γ (25,000 U·ml$^{-1}$) | 100 | -8.59±0.07 | (8) |

IFN-γ: gamma interferon; $IC_{50}$: concentration required to produce half-maximal relaxation.

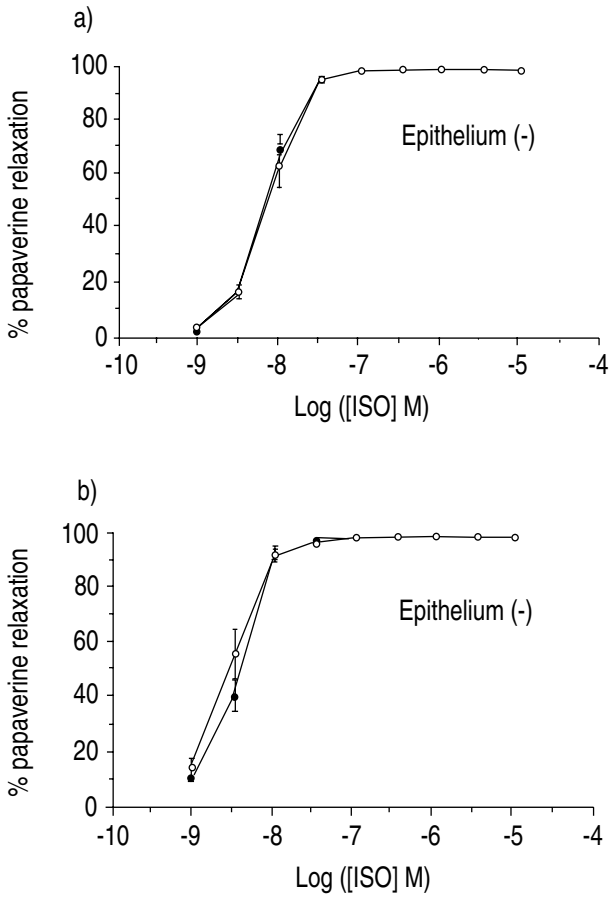


Fig. 2. – Concentration-response curves, showing arithmetic means with SEM, for isoproterenol (ISO) from tracheal strips without epithelium in the presence of 25,000 U·ml$^{-1}$ gamma interferon (IFN-γ) (○) and in control (●) for a) 1 hour and b) 5 hours. There is no significant shift in concentration response curves of strips incubated with IFN-γ from control.

*Examination of the specificity of IFN-γ*

The percentage of the cells which expressed MHC class II antigen in guinea pig peritoneal lavaged cells incubated with and without 1,000 U·ml$^{-1}$ IFN-γ were 25.6±1.2(SE)% and 4.6±4.5(SE)%, respectively. Incubation with human recombinant IFN-γ increased MHC class II antigen expression on guinea pig peritoneal lavaged cells. One hour incubation of tracheal strips with heat inactivated IFN-γ (25,000 U·ml$^{-1}$) showed no significant effect on ISO relaxation (table 7). One hour incubation with LPS (0.625 pg·ml$^{-1}$) also showed no significant effect on ISO relaxation (table 7).

Table 7. – Characteristics of the effects of IFN-γ* in isoproterenol relaxation (with epithelium)

| | Maximum relaxation % | Log($IC_{50}$) | (n) |
|---|---|---|---|
| Control | 100 | -7.81±0.09 | (9) |
| Heat-inactivated IFN-γ | 100 | -7.94±0.05 | (7) |
| LPS (0.625 pg·ml$^{-1}$) | 100 | -7.95±0.08 | (9) |
| IFN-γ + cycloheximide, 70 μM | 100 | -7.88±0.08 | (9) |
| Control + Indomethacin, 2 μM | 100 | -7.90±0.07 | (8) |
| IFN-γ + Indomethacin, 2 μM | 100 | -7.93±0.07 | (8) |
| Control + N$^{\omega}$-NAME, 30 μM | 100 | -7.96±0.10 | (9) |
| IFN-γ + N$^{\omega}$-NAME, 30 μM | 100 | -8.23±0.08* | (10) |

Strips were incubated for 1 h with the compounds listed. *: IFN-γ 25,000 u·ml. IFN-γ: gamma interferon; $IC_{50}$: concentration required to produce half-maximal relaxation LPS: lipopolysaccharide; N$^{\omega}$-NAME; N$^{\omega}$-nitro-L-arginine methyl ester; *: significantly different from control, $p<0.05$.

*Characteristics of the effect of IFN-γ on ISO relaxation*

The effects of cyclohexamide (70 μM), indomethacin (2 μM), and N$^{\omega}$-NAME (30 μM) are shown in table 7. ISO caused complete relaxation in preparations treated with cycloheximide, indomethacin, and N$^{\omega}$-NAME. For log($IC_{50}$)s, cycloheximide and indomethacin completely abolished the effect of IFN-γ (25,000 U·ml$^{-1}$) on ISO relaxation. However, the effect of IFN-γ on ISO relaxation was not affected by N$^{\omega}$-NAME.

## Discussion

There is increasing awareness of the pathophysiological significance of inflammatory mechanisms in asthma [1, 2]. In this process, as a final sequence, eosinophil accumulation and epithelial damage in the airways have been blamed for airway hyperresponsiveness, the hallmark of asthma [4, 17, 18]. On the other hand, in the field of immunology, a number of studies have demonstrated a disturbance of cell-mediated immunity in asthma, especially emphasizing the role of activated T-cells as a regulator of IgE production and as a conductor of airway inflammations [4, 19, 20]. In addition to these findings, recent developments in research have led to increased interest about the roles of cytokines in development of asthma and airway hyperresponsiveness [8]. Of these cytokines, IFN-γ might have a close relation to asthma and airway hyperresponsiveness.

Viral infection, a well-known stimulator of IFN-γ, has been known to make asthma symptoms worse and to induce airway hyperresponsiveness [10, 11].

In this study, we examined the effect of IFN-γ on airway strips and found that IFN-γ significantly increased the sensitivity to ISO relaxation without affecting the contracting response to KCl. Although 5 h incubation with 25,000 U·ml$^{-1}$ IFN-γ significantly decreased the reduction of maximum contraction by carbachol, the difference was small (-0.53g in control to -0.35g in IFN-γ treated tissue). These results raise the possiility that the main effect of IFN-γ on airway strips may be protective in terms of airway spasm. We also demonstrated that the effect of IFN-γ on ISO relaxation was completely abolished by the denudation of airway epithelium from the strips. These results suggest that IFN-γ may not act directly on smooth muscle but affect smooth muscle function through its effect on the cells which compose airway mucosa. Abolition of the effect of IFN-γ on ISO relaxation by cycloheximide suggests the requirement of protein synthesis for this effect.

Airway epithelium is known to produce several arachidonic acids metabolites including prostaglandins and leukotrienes. Nitric oxide (NO) is also released from airway epithelium. In this study, indomethacin (2 μM) completely abolished the effect of IFN-γ on ISO relaxation in the strips with intact epithelium, but N$^{\omega}$-NAME did not. These results suggest that the production and release of prostaglandin but not NO from airway epithelium may be related to the effect of IFN-γ.

Several previous studies demonstrated that the airway epithelium can produce prostaglandin $E_2$ ($PGE_2$) and this $PGE_2$ is partly responsible for the protective effect of airway epithelium on airway spasm. For this reason, $PGE_2$ may be the best possible candidate for the prostanoid which mediates the effect of IFN-γ on ISO relaxation. Speculation of the specific cell type in the airway epithelium responsible for the effect of IFN-γ was not possible from this study.

The finding that IFN-γ increases sensitivity of airway strips to ISO is in clear contrast to the concept proposed by Davis and associates [21]. They demonstrated that IFN-γ inhibits agonist-induced cyclic adenosine monophosphate (c-AMP) accumulation in human lymphocytes and suggested that interferon may play a role in inducing or potentiating bronchospasms. Considering the fact that viral infection worsens asthma and intensifies airway hyperresponsiveness [10, 11], this hypothesis sounds quite reasonable. However, IFN-γ has also been reported to increase c-AMP levels in the cells [22–24]. In addition, recent studies have revealed somewhat different aspects of the role of IFN-γ. IFN-γ has been known to down-regulate IgE production directly or by antagonizing the effect of IL4 [7]. Production of IFN-γ by leukocytes from asthmatic patients has been shown to be decreased when compared with that from healthy donors [12]. A recent report by Rousset and associates [14] also demonstrated that an enhanced IL-4 and reduced IFN-γ production are associated with the elevation of serum IgE levels observed in atopic individuals. These findings suggest IFN-γ may work against the worsening of asthma. Our results seem to be in agreement with such reports. Airway hyperreactivity induced by viral infection is speculated, partly due to deterioration of airway epithelial functions [25]. Airway smooth muscle of asthmatics is also reported to have reduced sensitivity to ISO [26, 27]. These facts, together with the results of this study, suggest that IFN-γ might work against hyperreactivity or worsening of asthmatic symptoms. However, this study was done only *in vitro* system, further *in vivo* studies are clearly required to confirm these speculations.

In this study, the effect of IFN-γ on airway smooth muscle was detectable from 1,000 U·ml$^{-1}$ (50 ng·ml$^{-1}$) for ISO relaxation. The concentrations of IFN-γ used in this study might be relatively high in comparison to the ordinary concentrations examined in other studies [22, 24]. However, stimulated peripheral lymphocytes have been reported to produce a large amount of IFN-γ. Lin *et al*. reported that $1\times10^6$ of peripheral lymphocytes from asthmatics produced more than 5,000 U·ml$^{-1}$ of IFN-γ [28]. Croll and associates also reported that stimulated lymphocytes can produce more than 1,000 U·ml$^{-1}$ of IFN-γ [29]. Although it is hard to estimate the local concentration of IFN-γ, the concentrations of IFN-γ used in this study may not be an unreasonable range. In addition, in this study, tracheal strips in the tissue baths, but not individually separated smooth muscle cells, were exposed to IFN-γ. In such a system, a relatively higher concentration of IFN-γ may be required to penetrate surrounding tissues and achieve the proper concentration at the smooth muscle cell level. Another reason for relatively higher concentration required to detect the effect might be the application of human recombinant IFN-γ on guinea pig airways. Questions for a specificity and a nonspecific effect due to high concentration of IFN-γ might be raised. However, IFN-γ used in this study increased the expressions of MHC class II antigen in guinea pig peritoneal lavaged cells. In addition, heat-inactivated IFN-γ and LPS did not show significant effects on ISO relaxation. These results may support the specificity of the action of IFN-γ in this study.

In summary, we investigated *in vitro* effects of IFN-γ on airway strips and found that IFN-γ increases sensitivity to ISO relaxation. IFN-γ seems to affect airway functions by inducing release of prostanoids from airway epithelial cells.

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