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Effects of Interleukin-18 on Diaphragm Muscle Contraction on Rats

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SASAKI, F., SHINDOH, C., NIWA, T., OHTAKA, T. and SHINDOH, Y. *Effects of Interleukin-18 on Diaphragm Muscle Contraction in Rats.* Tohoku J. Exp. Med., 2002, **196** (4), 269-280 —— To evaluate the role of interleukin (IL)-18 in endotoxin-induced diaphragm muscle deterioration, we studied three treatment groups, namely, a saline+endotoxin group, an IL-18+endotoxin group and an IL-18 only group using Wistar rats. Five minutes after saline or IL-18 (0.25 μg) injection, *E. coli* endotoxin (30 mg/kg) was injected intraperitoneally. In the saline+endotoxin group, the force-frequency curves by ANOVA ($p<0.01$), twitch tension (TT) and slope during contraction time (TT/CT) were significantly lower at 4 hours than those at 0 hours due to endotoxin ($p<0.01$ and $p<0.05$, respectively) and nitric oxide (NO) production had increased at 4 hours as shown by NADPH diaphorase staining. In the IL-18+endotoxin group, the decrement of the force-frequency curves in a higher frequency range (50-120 Hz), and the decrements of TT and TT/CT observed at 4 hours in the saline+endotoxin group were significantly prevented, and NO production was blocked at 4 hours. In the IL-18 only group, the force-frequency curves did not change except for fatigability, and NO production did not occur. It is suggested that IL-18 itself naturally blocks NO production, even when endotoxin coexists, resulting in the prevention of deterioration of diaphragm muscle contraction by endotoxin. ———— cytokine; endotoxin; NADPH diaphorase; nitric oxide

It is known that the injection of endotoxin induces a decrement in diaphragm muscle contractility and that this deterioration may be caused by a network of cytokines such as tumor necrosis factor-α (TNF-α), as well as by free radicals such as nitric oxide (NO) and oxygen-derived intermediates (Shindoh et al. 1995). Based on our previous studies of IL-10 and IL-13 (Taneda et al. 1998; Takahashi et al. 1999), we speculate that T helper type 2 (Th2)

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cells cytokines play protective roles in diaphragm muscle functioning after endotoxin injection into the peritoneal cavity. Because interleukin (IL)-18, originally called interferon-γ-inducing factor (IGIF), was cloned from the livers of mice treated with the bacterium *Propionibacterium acnes (P. acnes)* (Okamura et al. 1995), it induces toxic shock. It is involved in the development of T helper type 1 (Th1) cells and also in mechanisms of tissue injury in inflammatory reactions having a synergistic effect on IFN-γ production between IL-18 and IL-12 (Okamura et al. 1995). Although IL-12, natural killer cell stimulatory factor (NKSF), acts to enhance Interferon-γ (IFN-γ) production, resulting in augmentation of NK cell-mediated cytotoxicity (Kobayashi et al. 1989), we have previously reported an unexpected observation, namely, that IL-12 protects against endotoxin-induced diaphragm muscle deterioration (Nakahata et al. 2001). Thus, as is the case with IL-12, it is suggested that IL-18 can inhibit further deterioration of endotoxin-induced diaphragm muscle impairment, although this has not yet been substantiated. Elucidation of the roles of Th1 (IL-12, IL-18, etc.) and Th2 (IL-10, IL-13, etc.) cytokines in endotoxin-induced diaphragm dysfunction would not only contribute to the understanding of the patho-physiological mechanisms of sepsis, but might also be helpful in the development of new therapies for respiratory muscle failure related to sepsis.

In the present study, we examined the effects of the pre-injection of IL-18 on endotoxin-induced diaphragm muscle dysfunction by physiological measurements, examined whether IL-18 itself has any effects on diaphragm muscle, and performed nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase staining as a marker of NO production. It was observed that IL-18 prevents endotoxin-induced diaphragm muscle deterioration by blocking NO production and that IL-18 in the absence of endotoxin neither changed force-frequency curves nor led to the production of NO.

Materials and Methods

Animal preparation

Using Wistar rats weighing 202.4 ± 7.4 g (Charles River Japan, Yokohama), experiments were performed on a total 48 animals divided into 4 groups. Group 1, the saline+endotoxin group, was given 0.5 ml of saline intravenously via the tail vein, followed by an intraperitoneal injection of *E. coli* endotoxin (30 mg/kg, 055 : B5, Sigma Chemical Co., St. Louis, MO, USA) in 0.5 ml of saline ($n=12$) 5 minutes later. Group 2, the IL-18+endotoxin group, was given recombinant human IL-18 (0.25 μg, B001-5, Medical and Biological Laboratories (MBL) Co., Ltd., Nagoya) suspended in 0.5 ml of saline intravenously via the tail vein, followed by an intraperitoneal injection of *E. coli* endotoxin (30 mg/kg) in 0.5 ml of saline ($n=12$) 5 minutes later. Group 3, the endotoxin+IL-18 group, received an intraperitoneal injection of *E. coli* endotoxin (30 mg/kg) in 0.5 ml of saline followed by IL-18 (0.25 μg) suspended in 0.5 ml of saline intravenously via the tail vein ($n=12$) 5 minutes later. For the analysis of diaphragm muscle contractile properties, force-frequency curves, twitch kinetics and fatigability were measured immediately after (0 hours) and at 4 hours after injection of endotoxin in each experimental group ($n=6$, respectively). The times of measurements were selected according to a previous study (Shindoh et al. 1995), and IL-18 effects were analyzed by comparison of data at 0 hours and at 4 hours after endotoxin administration. In addition, Group 4, the IL-18 only group, was given only recombinant human IL-18 (0.25 μg, B001-5, MBL Co., Ltd.) suspended in 0.5 ml of saline intravenously via the tail vein, and force-frequency curves, twitch kinetics and fatigability were measured at 0 hours and 4 hours ($n=6$, respectively). Written approval was obtained from the Tohoku University Animal Facility.

Measurements of muscle contraction

Two muscle strips (3-4 mm wide) were dissected from the right and left hemidiaphragm under diethyl ether anesthesia and mounted in separate organ baths containing Krebs-Henseleit solution oxygenated with a 95% O_2-5% CO_2 gas mixture (37.0±0.5°C, pH 7.40±0.05). The composition of the aerated Krebs-Henseleit solution in mEq/liter was as follows: Na^+, 153.8; K^+, 5.0; Ca^{2+}, 5.0; Mg^{2+}, 2.0; Cl^-, 145.0; HCO_3^-, 15.0; HPO_4^{2-}, 1.9; SO_4^{2-}, 2.0; glucose, 110 mg%; d-tubocurarine, 10 μM; and regular crystalline zinc insulin, 50 U/liter. Both muscle strips were simultaneously stimulated with supramaximal currents of 200-250 mA (i.e., 1.2 to 1.5 times the current required to elicit maximal twitch tension, pulse duration of 0.2 milliseconds) by a constant current stimulus isolation unit (SS-302J, Nihon Kohden, Tokyo) driven by a stimulator (SEN-3201, Nihon Kohden). The elicited tensions were measured by a force transducer (UL-100GR, Minebea Co., Fujisawa). The length of each muscle strip was changed by moving the position of the force transducer with a micrometer-controlled rack and pinion gear (accuracy of displacement, 0.05 mm, Mitsutoyo Co., Kawasaki), and measured with a micrometer in close proximity to the muscle. The optimal length of the muscle (Lo) was defined as the muscle length at which twitch tension development was maximal, and this Lo was maintained in the following measurements.

The diaphragm force-frequency relationship was assessed by sequentially stimulating muscles at 1, 10, 20, 30, 50, 70, 100 and 120 Hz. Each stimulus train was applied for approximately 1 second, and adjacent trains were applied at approximately 10-second intervals. The tensions of both muscle strips were recorded by a hot-pen recorder (RECTI-HORIZ-8K, San-ei, Tokyo). The force-frequency curves obtained from the groups studied were displayed as elicited tensions (kg/cm^2) on the Y-axis and stimulating frequencies on the X-axis.

Twitch contraction was elicited by a single pulse stimulation (duration of pulses, 0.2 milliseconds), and the trace of the twitch contraction was recorded at high speed (10 cm/seconds). The twitch kinetics were assessed by (I) twitch tension (TT, peak tension of twitch contraction, kg/cm^2), (II) contraction time (CT, the time required to develop peak tension, milliseconds) and (III) half-relaxation time (HRT, the time required for peak tension to fall by 50%, milliseconds) during a single muscle contraction. For analysis of contractile velocity of twitch contractions, TT/CT (slope during contraction time) and (1/2 TT)/HRT (slope during half-relaxation time) were calculated from the curve of the twitch contraction trace.

Muscle fatigability was then assessed by examining the rate of the fall of tension over a 5-minute period of rhythmic contraction. Rhythmic contraction was induced by applying trains of 20 Hz stimuli (train duration, 0.3 seconds; rest duration, 0.7 seconds) at a rate of 60 train/min. Muscle fatigability was expressed as a percentage of the final remaining tension (%) from the initial tension. After completion of this protocol, the muscle strip was removed from the bath and weighed.

NADPH diaphorase histochemistry

NADPH diaphorase histochemistry was performed using the diaphragm muscles of 0 hours and 4 hours in the saline+endotoxin and IL-18+endotoxin groups, and in the IL-18 only group. The diaphragm samples were quickly excised from the remaining part of the costal diaphragm muscle (after dissection of the muscle strips used for measurement of muscle contractile properties), immersed in isopentane (Wako Pure Chemical Industries Ltd., Osaka) that had been cooled in liquid nitrogen, and embedded in mounting medium (OCT compound, Miles Inc., Elkhart, IN, USA). Diaphragm sample tissues were sectioned at 10 μm

with a cryostat (BRIGHT Instrument, Huntingdon, UK) kept at −20°C, mounted on chrome alum gelatin-coated glass slides, and immersed in 0.3% Triton X-100 containing phosphate buffer. For histochemical reaction for NADPH diaphorase, the sections were submerged for 60 minutes at 37°C in freshly prepared 1.0 mM β-NADPH (Oriental Yeast Co., Tokyo) and 0.2 mM nitroblue tetrazolium (Wako Pure Chemical Industries Ltd., Osaka) in 100 mM Tris-HCl buffer (pH 8.0), containing 0.2% Triton X-100 (Dawson et al. 1991). The reaction was terminated by rinsing the sections in phosphate buffer saline (PBS). The sections were covered with a mixture of glycerol and PBS (2 : 1), and photographed with an Olympus microscope using color reversal film (Sensia II, Fujifilm, Tokyo).

Data analysis

The cross-sectional area of the strip was calculated by dividing the muscle mass by the product of the strip muscle length and muscle density (1.06 g/cm^3) (Close 1972), and tension was calculated as force per unit area (kg/cm^2). Data obtained from both halves of the diaphragm in one animal were averaged; therefore, the number of samples used was $n=6$ (animals) per treatment/time point for force-frequency curves, twitch kinetics and fatigability. The mean values of tension for each frequency in force-frequency curves, twitch kinetics and fatigability were compared by Students t-test. To compare the entire configuration of each force-frequency curve at 0 and 4 hours of each group, analysis of variance (ANOVA) with Fishers PLSD (Protected Least Significant Difference) *post hoc* test was performed. Data are presented as means±S.E. Comparisons with a p-value of less than 0.05 were considered to be statistically significant.

RESULTS

Changes of force-frequency curves

In the saline+endotoxin group, the tensions of force-frequency curves at 4 hours significantly decreased from those at 0 hours at each frequency (1, 10, 30, 50, 70, 100 and 120 Hz: $p<0.01$; 20 Hz: $p<0.001$, respectively). The entire configuration of the force-frequency curves at 4 hours (1.62±0.06 kg/cm^2 as a peak) were significantly lower than that at 0 hours (1.98±0.08 kg/cm^2 as a peak) by ANOVA ($p<0.01$) (Fig. 1A). In the IL-18+endotoxin group, there were significant decreases at 10, 20 (both, $p<0.01$) and 30 Hz ($p<0.05$), but no significant changes at higher frequencies. Overall, therefore, there were no significant changes in the entire configuration of force-frequency curves between 0 hours (1.94±0.07 kg/cm^2 as a peak) and 4 hours (1.87±0.06 kg/cm^2 as a peak) (Fig. 1B). In the endotoxin+IL-18 group, there were no significant changes of force-frequency curves between 0 hours (2.03±0.06 kg/cm^2 as a peak) and 4 hours (1.95±0.08 kg/cm^2 as a peak) (Fig. 1B′). Thus, comparison of the entire configurations of the force-frequency curves at 4 hours of the saline+endotoxin group and at 4 hours of the IL-18+endotoxin group indicated that there was a significant inhibition of IL-18 as shown by ANOVA ($p<0.05$). This indicates that IL-18 prevented deterioration in the higher ranges of force-frequency curves by endotoxin at 4 hours after injection.

Changes of twitch kinetics and fatigability

Regarding twitch tension, in the saline+endotoxin group, it was significantly lower at 4 hours (0.40±0.02 kg/cm^2) than at 0 hours (0.51±0.03, $p<0.01$) (Fig. 2A). In the IL-18+endotoxin group, there was no significant change between 0 hours and 4 hours (0.54±0.03, 0.45±0.03 kg/cm^2, respectively), but there was a significant increase in this group at 0 hours as compared with that of the saline+endotoxin group at 4 hours ($p<0.01$). As shown in Figs. 2B, 2C and 2D, contraction time, half-relaxation time and fatigability of each group did not show significant changes between at 0

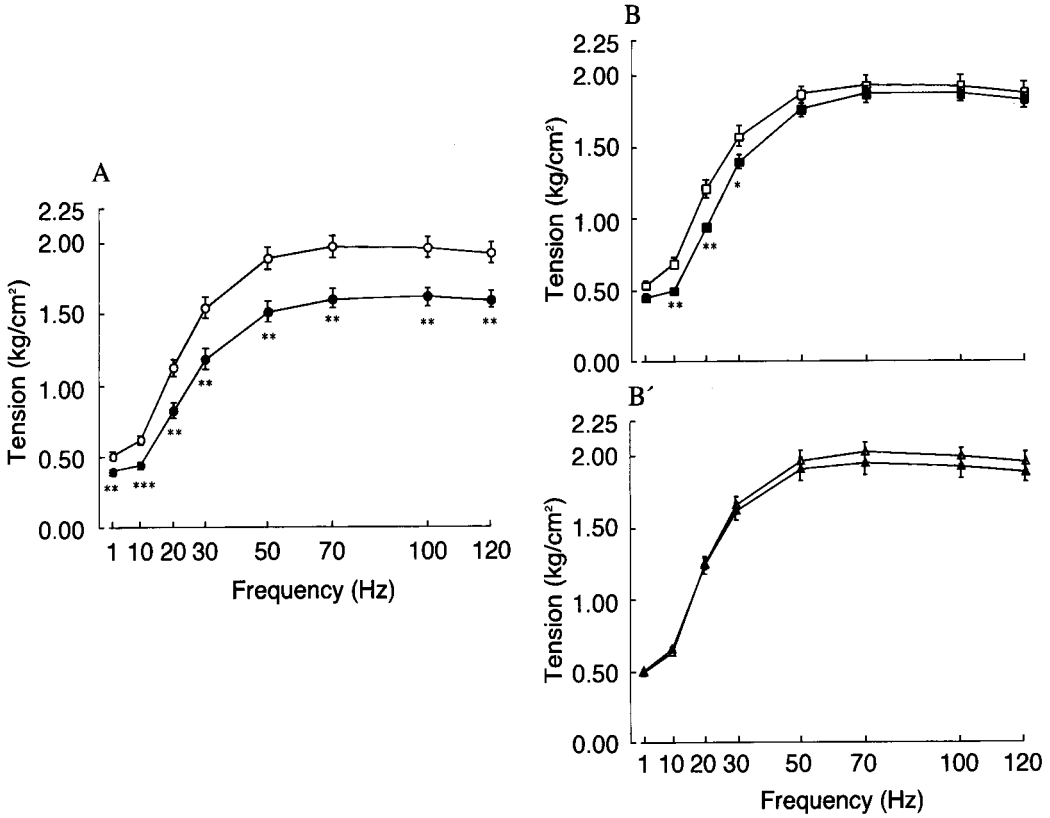

Fig. 1. Changes of force-frequency curves. A, 0 hours (○) and 4 hours (●) in the saline+endotoxin group; B, 0 hours (□) and 4 hours (■) in the IL-18+endotoxin group; B′, 0 hours (△) and 4 hours (▲) in the endotoxin+IL-18 group.
$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, compared to 0 hour in each group.

hours and 4 hours.

Fig. 3 summarizes the slopes during contraction and half-relaxation times of the twitch contractile trace in each group for analysis of contractile velocity. As shown in Fig. 3A, TT/CT of the saline+endotoxin group at 4 hours (0.0111 ± 0.0008 kg/cm^2/milliseconds) was significantly less than that of the this group at 0 hours (0.0142 ± 0.0009, $p<0.05$) and that of the IL-18+endotoxin group at 0 hours (0.0139 ± 0.0008, $p<0.05$). As shown in Fig. 3B, there were no significant changes in the (1/2 TT)/HRT of each group. Thus, it can be seen that IL-18 inhibited the decrease of TT/CT of the saline+endotoxin group at 4 hours.

Effects of IL-18 only

Fig. 4 shows the force-frequency curves at 0 hours and at 4 hours in the IL-18 only group. There were no significant differences between the two points of time, nor was the entire configuration of the force-frequency curves significant by ANOVA.

Fig. 5 summarizes the changes in twitch kinetics at the two points of time. IL-18 itself did not result in significant changes in twitch tension (Fig. 5A), contraction time (Fig. 5B), half-relaxation time (Fig. 5C), TT/CT (Fig. 5E) and (1/2 TT)/HRT (Fig. 5F). However, there was a significant decrease in the fatigability at 4 hours (Fig. 5D) ($p<0.05$). It seems that IL-18 had very little effect on single twitch contraction, but that repetitive stimulation affected fatigability measured at 5 minutes.

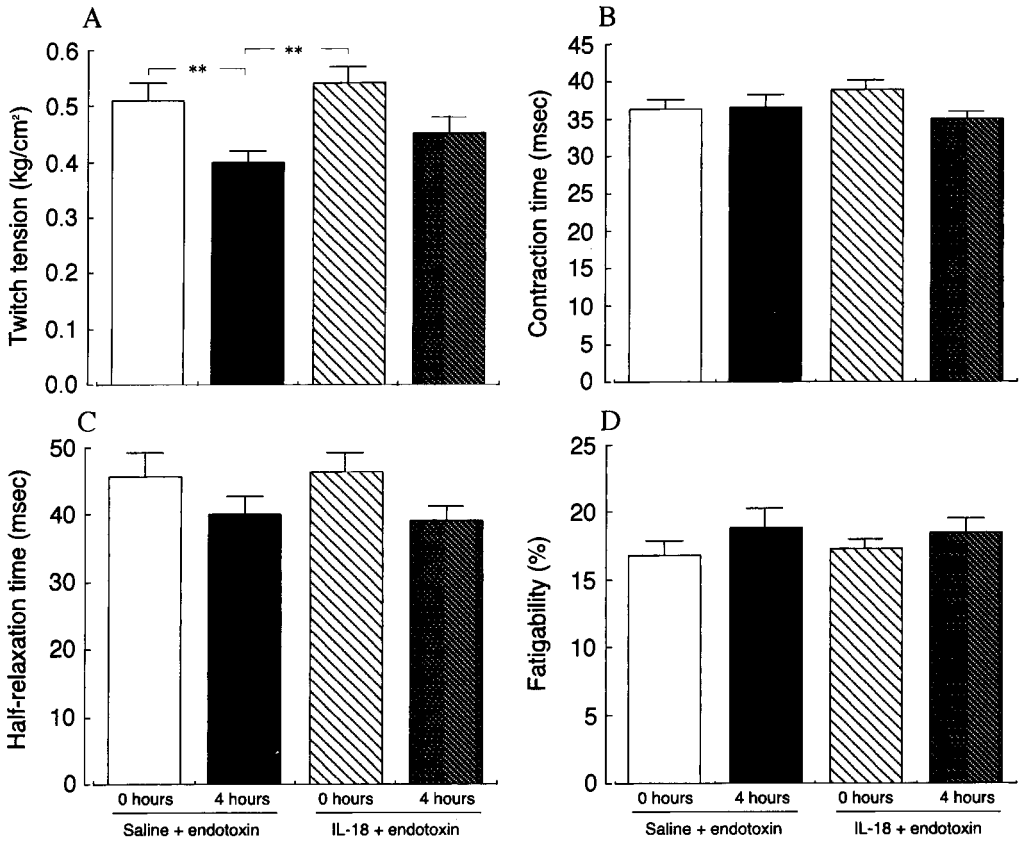

Fig. 2. Changes of twitch kinetics and fatigability. A, twitch tension; B, contraction time; C, half-relaxation time; D, fatigability. Open column (0 hours) and closed column (4 hours) in the saline+endotoxin group; slashed column (0 hours) and densely slashed column (4 hours) in the IL-18+endotoxin group.
$^{**}p<0.01$, compared in each group.

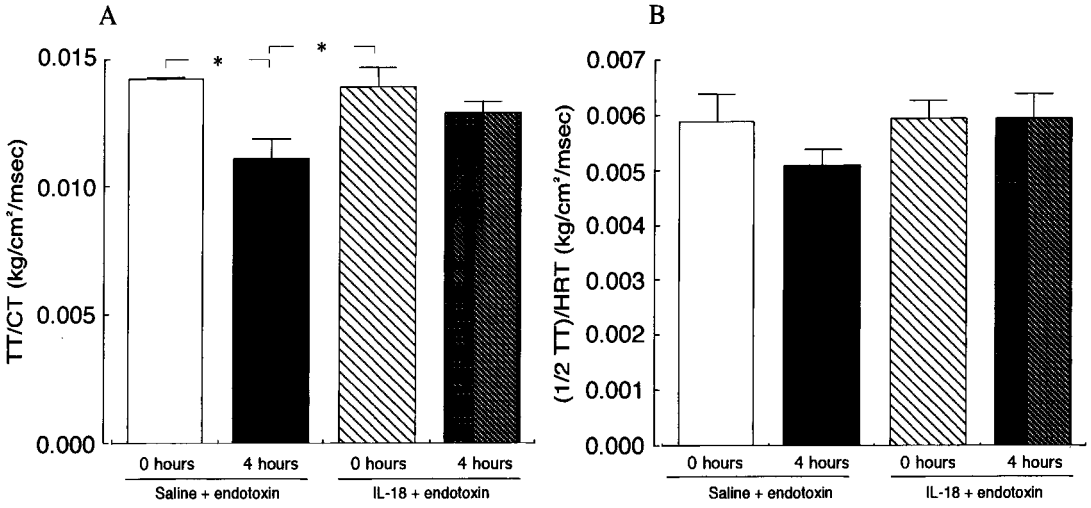

Fig. 3. Mean changes of slopes of twitch contraction trace in each group. A, slopes during contraction time (TT/CT); B, slopes during half-relaxation time ([1/2 TT]/HRT).
$^{*}p<0.05$, compared in each group.

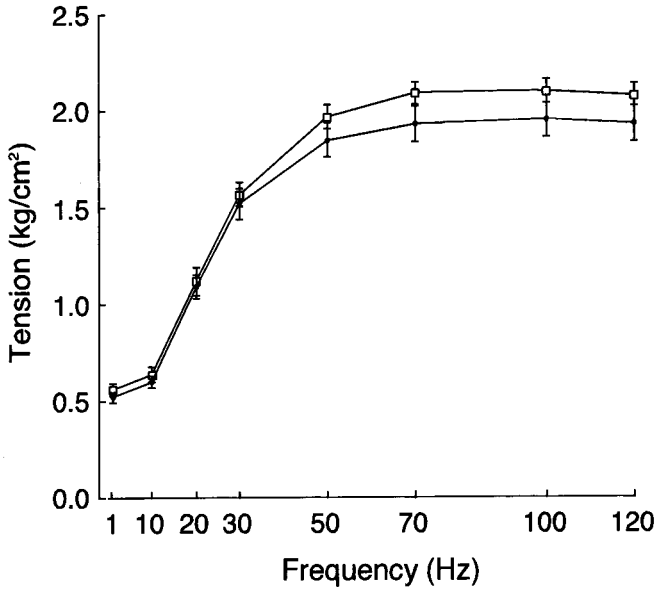

Fig. 4. Force-frequency curves of IL-18 only group. A, 0 hours (□); B, 4 hours (●).

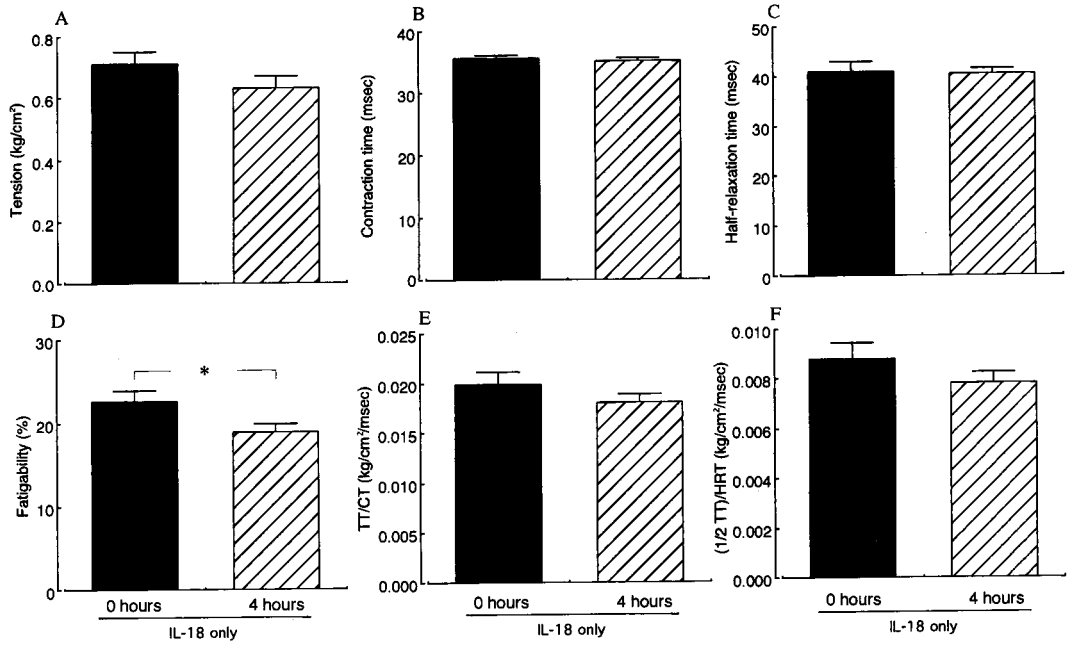

Fig. 5. Changes of twitch kinetics, fatigability, and slopes of twitch contraction trace in IL-18 only group. A, twitch tension; B, contraction time; C, half-relaxation time; D, fatigability; E, slopes during contraction time (TT/CT); F, slopes during half-relaxation time ([1/2 TT]/HRT).

*$p < 0.05$, compared in each group.

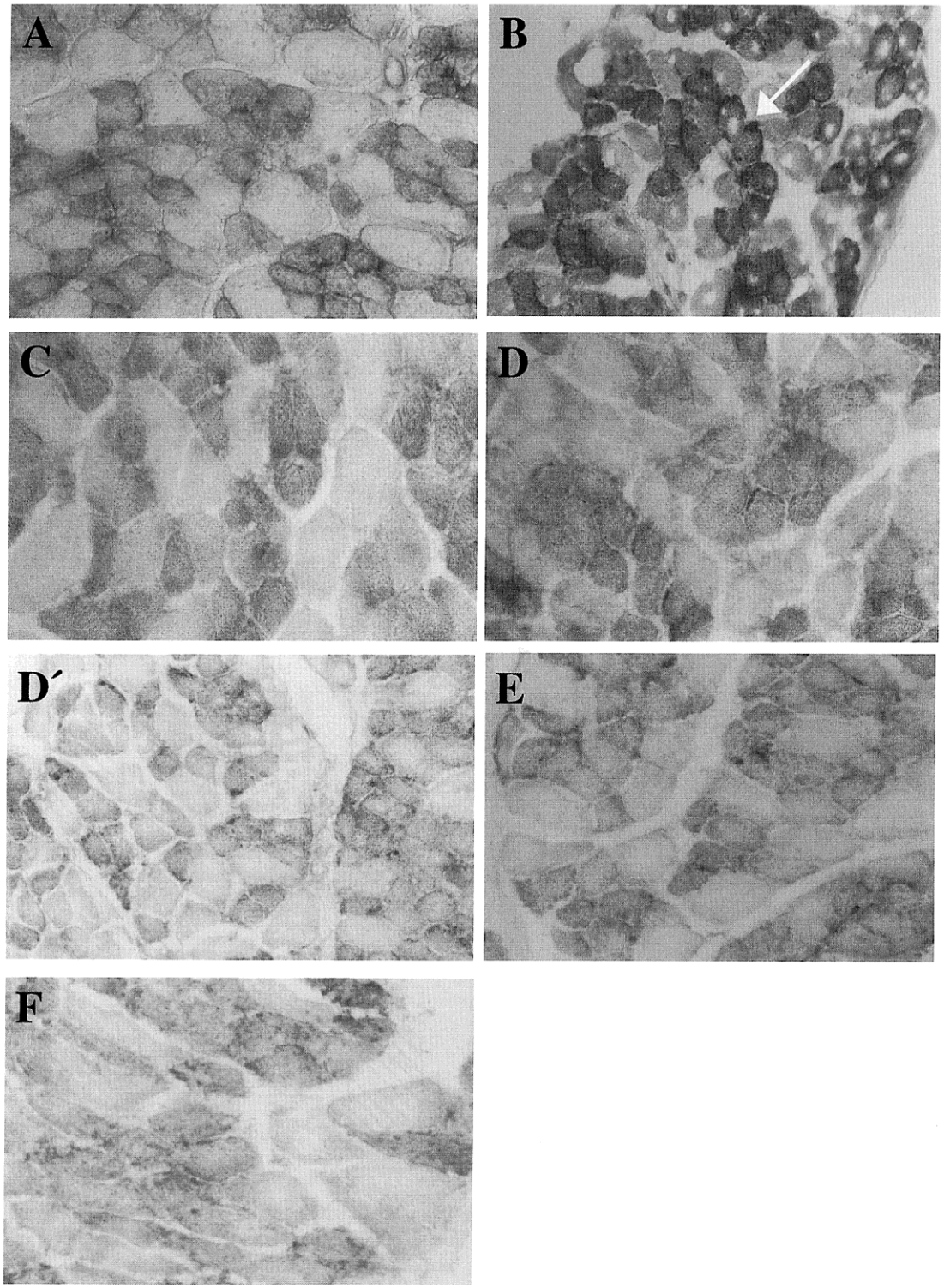

Fig. 6. NADPH diaphorase staining of diaphragm muscles. A and B, at 0 hours and 4 hours, respectively, in the saline+endotoxin group; C and D, at 0 hours and 4 hours, respectively, in the IL-18+endotoxin group; D′, at 4 hours in the endotoxin+IL-18 group; E and F, at 0 hours and at 4 hours, respectively, in the IL-18 only group. White arrow indicates positive stained muscle fibers (×200).

NADPH diaphorase staining

Fig. 6 shows representative photographs of the NADPH diaphorase staining in each group. In the saline+endotoxin group, muscle fibers were only slightly stained at 0 hours (Fig. 6A); however, there were some strongly stained muscle fibers (positive: white arrows) at 4 hours (Fig. 6B). On the other hand, the IL-18+endotoxin group did not show strongly stained muscle fibers at either 0 hours (Fig. 6C) or 4 hours (Fig. 6D). The endotoxin+IL-18 group did not show significant staining at 4 hours (Fig. 6D′) either. These findings indicate that although endotoxin induces more NO production in muscle fibers at 4 hours than that at 0 hours, IL-18 inhibits NO production induced by endotoxin in the diaphragm muscle at 4 hours.

At both 0 hours (Fig. 6E) and 4 hours (Fig. 6F), the IL-18 only group did not show significant positive NADPH diaphorase staining when compared with that of the saline+endotoxin group at 4 hours (Fig. 6B). Thus, IL-18 by itself does not appear to induce NO production in diaphragm muscle.

DISCUSSION

In the present study, IL-18 prevented the decrease of the higher frequency range of force-frequency curves induced by the endotoxin intraperitoneal injection and was also found to inhibit NO production at 4 hours after endotoxin administration. In addition, IL-18 itself did not change force-frequency curves except for fatigability, and also inhibited NO production at 4 hours, thus preventing diaphragm muscle deterioration. Taken together, these findings suggest that IL-18 itself blocks iNOS expression by nature and that the inhibition of iNOS expression by IL-18 is stronger than the induction of iNOS by coexisting endotoxin.

IL-18, originally called IFN-γ-inducing factor (IGIF), is also produced by activated macrophages such as Kupffer cells (Okamura et al. 1995). The major activity of IL-18 is induction of IFN-γ production from T cells and NK cells (Okamura et al. 1995) and it enhances of their cytotoxicity through a Fas ligand (FasL)-mediated mechanism, particularly in the presence of IL-12 (Tsutsui et al. 1996). It acts as a co-stimulatory factor for Th1 clones stimulated with Ag on antigen presenting cells (APC), immobilized anti-CD3 mAb, or Con A, resulting in an increase in IFN-γ production and a proliferation of Th1 clones. Unlike IL-12, IL-18 does not drive Th1 development, but potentiates IL-12-driven Th1 development (Robinson et al. 1997). IL-18 selectively enhances the FasL-mediated cytotoxicity of cloned murine Th1 cells, but not Th0 or Th2 cells. Anti-IFN-γ antibody (Ab) does not block the IL-18-induced cytotoxicity of Th1 cells, nor does IFN-α, IFN-γ or TNF-α augment the cytotoxicity of Th1 cells mediated by IL-18. IL-12 has also been found to enhance the FasL-mediated cytotoxicity of Th1 cells, suggesting that Th1 cells possess receptors for both cytokines, although these cytokines can act via different pathways (Dao et al. 1996). In addition, IL-12 is mainly produced by macrophages and dendritic cells and induces naive $CD4^+$ T cells to develop into Th1 cells (Trinchieri 1995), whereas IL-18 has no such capacity (Matsui et al. 1997). Although IL-18 and IL-12 both contribute to INF-γ induction, there are some differences in their actions. For example, IL-18 requires the existence of IL-12 to induce INF-γ, but IL-12 can induce INF-γ by itself. Therefore, it can be said that IL-18 induces interferon (IFN)-γ synthesis and synergizes with IL-12 in T helper type 1 (Th1) but not Th2 cell development. IL-18 receptor (IL-18R) is selectively expressed on murine Th1 but not Th2 cells, and anti-IL-18R antibody inhibits IL-18-induced IFN-γ production by Th1 clones in vitro, and reduces local inflammation and lipopolysaccharide-induced mortality in mice (Xu et al. 1998). On this point, IL-18 and IL-12 are both Th1 cytokines, although there are some differences between

them.

Recently, it has been reported that IL-18 requires cleavage by caspase-1 to become active and that it induces naïve $CD4^+$ T cells to develop into IL-4 producing cells in the production of immunoglobulin E (IgE) (Yoshimoto et al. 2000). If so, we speculate that IL-18 may induce Th2 cytokines, such as IL-4, IL-10 and IL-13, and that these cytokines may inhibit the deterioration of diaphragm muscle contraction and NO production due to endotoxin as previously reported (Taneda et al. 1998; Takahashi et al. 1999). Therefore, it seems that the differences of IL-12 and IL-18 may be the result of differences of signal transduction pathways. Furthermore, the dosage of IL-18 ranged from 0.2 to 20 ng in vitro, an amount which can induce IL-4 around 80 ng/ml (Yoshimoto et al. 2000). The dosage of IL-18 (0.25 μg) appears to have been sufficient to induce Th2 cytokines in this study.

It was noteworthy that IL-18 itself did not induce NO production in the diaphragm muscles at 4 hours and that the inhibition of NO production was not impaired by endotoxin stimulation. IL-18 and IL-12 utilize different signal transduction pathways, and IL-18 as well as IL-12 are endogenously released through interaction between Th1 cells and spleen cell APC in the presence of specific Ag and regulate IFN-γ production (Kohno et al. 1997).

Generally speaking, when endotoxin is administered to heat-killed *P. acnes*-primed BALB/c nude mice, they develop endotoxin-induced liver injury. Such injury can be prevented by treatment with an Ab against IL-18, and IL-18 activates both TNF-α and FasL-mediated hepatocytotoxic pathways in endotoxin-induced liver injury (Tsutsui et al. 1997). Endotoxin has been found to induce IL-18 in the serum of *P. acnes*-primed wild-type C57BL/6 mice, while IFN-γ and IL-18 were not observed in the serum of *P. acnes*-primed caspase-1-deficient ($ICE^{-/-}$, interleukin-1β-converting enzyme, recently termed caspase-1) mice (Gu et al. 1997). The lack of multiple proinflammatory cytokines in $ICE^{-/-}$ mice may account for their protection from septic shock, and, therefore, ICE processing of pro-IL-18 and IFN-γ production may be central events in the pathogenesis of sepsis. Thus, mice lacking IFN-γ or its receptor are resistant to endotoxin shock (Gu et al. 1997). From the above-mentioned reports, it is speculated that IL-18 also contributes to the induction of endotoxic deterioration of diaphragm muscle contractions. However, no significant deteriorative effects of IL-18 itself were observed in the present study. Actually, the findings of the previous reports and those of the present study are contradictory. We suggest that these contradictions regarding diaphragm deterioration are strongly related to NO production in the diaphragm muscle fibers.

In the present study, NADPH diaphorase histochemistry indicated that NO production resulted from saline + endotoxin administration, but not from administration of IL-18 alone in the muscle fibers of the control group at 4 hours. The expression of inducible nitric oxide synthase (iNOS) mRNA has been reported to be induced by endotoxin (Liu et al. 1993), and mice deficient in the iNOS gene are more resistant to LPS-induced acute lung injury than are wild-type mice (Kristof et al. 1998). We have also reported that TNF-α mRNA is expressed in diaphragm muscle cells after endotoxin administration (Shindoh et al. 1995) and that IL-10 (Taneda et al. 1998) and IL-13 (Takahashi et al. 1999) have a protective effect against endotoxin-induced diaphragm muscle deterioration by blockage of NO production. IL-10 and IL-13 are classified as cytokines induced by Th2 cells, which are considered to act protectively against cytotoxic cytokines in immunology. The results of IL-10 and IL-13, therefore, are very consistent with the immunological observations. On the other hand, we have recently shown that IL-12 by itself induced NO production (Nakahata et al. 2001). This previous

study showed that IL-12 itself induces NO production positively, but inhibits NO production when endotoxin is coexistent. Although IL-12 augments the production of TNF-α and IFN-γ and induces cell death as an immunological function (Trembleau et al. 1995; Trinchieri 1995), we speculate that there is an interference between IL-12 and endotoxin in the diaphragm muscle. Since IL-12, like IL-18, is classified as Th1, it might be expected to induce NO production in the diaphragm muscle fibers. However, the present finding of no induction of NO by IL-18 seems contrary to previous observations. Rather, the present study showed that IL-18 actually inhibited the decrease of force-frequency curves induced by endotoxin and did not induce NO production in the absence of endotoxin. This may be explained by the phenomenon that IL-18 induces Th2 cytokines such as IL-4, IL-10 and IL-13 as mentioned above. Therefore, we suggest that the present results are interesting findings regarding the reciprocal functions between IL-18 and IL-12 cytokines.

In conclusion, IL-18 has a protective effect for diaphragm muscles in a septic animal model and appears to block iNOS expression induced by endotoxin. Although the protective effects of IL-18 on diaphragm muscle after endotoxin administration have been observed only in an animal model, it is suggested that IL-18 is a candidate for treatment of patients with respiratory muscle failure caused by sepsis. In addition, comparing the possible usefulness of IL-18 with that of IL-12 for the clinical treatment of patients with sepsis, IL-18 is recommended since it has almost no deteriorative effect on respiratory muscle by itself and is suggested to be much safer than IL-12.

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