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Short communication

Effect of interleukin-12 level augmented by Kakkon-to, a herbal medicine, on the early stage of influenza infection in mice

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Abstract

Oral administration of Kakkon-to (KT) (5.0 mg per mouse three times daily), a herbal medicine, for 8 days from 1 day before influenza virus infection exhibited therapeutic efficacy in the infected mice. The effect of KT-treatment on the levels of cytokines, especially interleukin (IL)-12, was evaluated in the early phase of infection. KT was significantly effective in reducing the weight loss of infected mice, prolonging their survival times and reducing mortality. In infected mice administered with KT, virus yield in the bronchoalveolar lavage fluid (BALF) of lungs was significantly lower than that in the control on day 3 after infection. On day 2 after infection, only the level of IL-12 in the BALF increased significantly in KT-administered mice as compared with the control. Thus the significant enhancement of IL-12 correlated with the reduction of virus yields in BALF in the early phase of infection. This suggested a key role of IL-12 in the alleviation of influenza in the KT-treated mice.

Keywords: Influenza; Cytokines; Medicinal herb; Interleukin-12

Interleukin (IL)-12 has been shown to contribute to the development and activation of the innate immune response and the inhibition of virus replication in the early phase of influenza infection in mice (Monteiro et al., 1998). We have previously shown that the augmentation of IL-12 production or administration of supplementary IL-12 at the optimal dose and timing in the respiratory tract of influenza virus-infected mice is significant in reducing virus yield in the bronchoalveolar lavage fluid (BALF) in early influenza infection and played a role in the alleviation of influenza (Tsurita et al., 2001). The augmentation of IL-12 level in the early phase of influenza infection has been verified to be important in alleviation of the infection.

Cytokines, such as IL-12 inducing Th1 immune response, interferon (IFN)-γ as a Th1 cytokine, IL-4 and -10 as Th2 cytokines and IL-1α, IL-1β, IL-6 and tumor necrosis factor (TNF)-α as in-

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flammatory cytokines, have been shown to be produced locally and systemically in influenza infection models in animals (Baumgarth et al., 1994; Baumgarth and Kelso, 1996; Conn et al., 1995; Hennet et al., 1992; Monteiro et al., 1998; Peper and Van Campen, 1995; Swiergiel and Dunn, 1999; Van Reeth, 2000). These cytokines modified the course of influenza virus infection, although the administration of IL-4, IL-12 and antibodies to TNF-α and IL-6 to influenza virus-infected mice did not influence their mortality (Moran et al., 1996; Kostense et al., 1998; Peper and Van Campen, 1995; Swiergiel and Dunn, 1999).

Traditional medicines have been used in the treatment of influenza infection in the Asian countries. It is important to understand the mechanism of their action, if they show the beneficial effects. In this aspect, we have documented their efficacies in animal models and clarified the mechanism of action in herpes simplex virus infection and influenza infection (Nagasaka et al., 1995; Kurokawa et al., 1996a,b). Further the active compounds in traditional medicines have been identified and reproduced the efficacies of herbal medicines in animal infection models (Kurokawa et al., 1998a,b, 1999, 2001). In an influenza virus-intranasal infection model in mice, Kakkonto (KT), a herbal medicine, has been shown to be effective in reducing fever production by suppressing the rise of IL-1α production, the severity of pneumonia and the mortality, although they did not exhibit direct anti-influenza virus activity in vitro (Kurokawa et al., 1996b, 1998b). In these cited studies KT showed therapeutic activity against influenza infection in mice.

In this study, we examined IL-12 and Th1- and Th2-cytokine levels in the BALF of lungs and serum in influenza virus-infected mice administered orally with a dose of KT that exhibited therapeutic efficacy. The augmentation of IL-12 level by KT in the early phase of influenza was shown to be associated with the alleviation of signs of the disease. This was consistent with our previous results (Tsurita et al., 2001) and confirmed that IL-12 production in the early phase was an important factor controlling the alleviation of influenza.

KT, a dried herbal medicine, is composed of seven medicinal herbs as described previously (Nagasaka et al., 1995; Kurokawa et al., 1996b). This herbal medicine was supplied from Kanebo Ltd., Tokyo, or Tsumura Co., Tokyo, Japan. KT was suspended in distilled water at 20 mg/ml and then used for oral administration to mice (Kurokawa et al., 1996b).

Female DBA/2 Cr mice (6-week-old, 17–19 g, Sankyo Labo Service Co., Ltd., Japan) were intranasally infected or mock-infected with 10^3 plaque forming units of influenza virus [A/PR/8/34 (H1N1)] under ether anesthesia (Kurokawa et al., 1996a). KT or water was administered orally by gavage to mice three times daily, at approximately 8 h intervals, for 8 days starting a day before infection. The dose of KT used in this study was 5.0 mg/0.25 ml × 3 per mouse per day, which has been shown to be effective in reducing severity of pneumonia and prolonging survival times in mice (Kurokawa et al., 1996b). The body weights of infected mice were measured daily. BALF was prepared from six mice in each group on days 2 and 3 after infection and the virus titers of BALF were determined by the plaque assay using Madin–Darby canine kidney cells as described previously (Kurokawa et al., 1996b). On days 2, 4 and 6 after infection, BALF and serum were prepared from four to six mice in each group for the determination of cytokine-concentrations (Kurokawa et al., 1996a). The levels of cytokines in the BALF or serum were determined by using the enzyme-linked immunosorbent assay (ELISA) kits (Amersham Pharmacia Biotech Inc., Piscataway, USA) according to the manufacturer's instructions.

Efficacy of KT was confirmed in an intranasal influenza virus infection model in mice as shown in Fig. 1. The body weight of infected mice decreased markedly later than 2 days after infection. However, KT significantly delayed the decrease of body weight in the early phase of infection ($P < 0.05$, Fig. 1A), prolonged the survival times of infected mice and reduced mortality ($P < 0.05$, Fig. 1B). Virus yield in the BALF of lungs of KT-administered mice was significantly lower than that in the control on day 3 after infection ($P < 0.05$, Fig. 1C). KT was effective in reducing virus yield in the

BALF and the weight loss of infected mice after infection and in alleviating the infection.

Levels of IL-12, IFN-γ, IL-4 and IL-10 were examined in the BALF and serum of influenza virus-infected mice administered with KT on days 2, 4 and 6 after infection. In comparison with mock-infected mice, the levels of IL-4 and -10 and IFN-γ rose after infection in the BALF of infected mice administered with water, especially on day 6, but marked change was not observed in the levels of IL-12 after infection (Fig. 2A). In infected mice administered with KT, changes in the levels of IL-4 and -10 and IFN-γ after infection were similar to those in infected mice administered with water. However, the level of IL-12 on day 2 was significantly higher than that of infected mice administered with water ($P < 0.05$). In BALF, the level of IL-12 was increased by KT in the early phase of influenza infection.

In comparison with the sera of mock-infected mice (Fig. 2B), the levels of IL-10 and IFN-γ increased significantly on day 6 after infection in infected mice administered with water, but no significant difference was observed in the levels of IL-4. In infected mice administered with KT, the level of IL-4 was significantly lower on day 6 than that in infected mice administered with water, although there was no significant difference in the levels of IL-10 and IFN-γ between KT- and water-administered mice. IL-12 was less than detectable level in all sera examined. The levels of IFN-γ increased markedly on day 6 in the infected mice. In serum, no remarkable changes were observed in the levels of IL-10, IFN-γ and IL-12 by different treatments.

KT modified cytokine production locally and systemically in influenza virus-infected mice. KT might locally induce Th1 immune response in the early phase of infection and promote cell-mediated immunity resulting in alleviation of the infection.

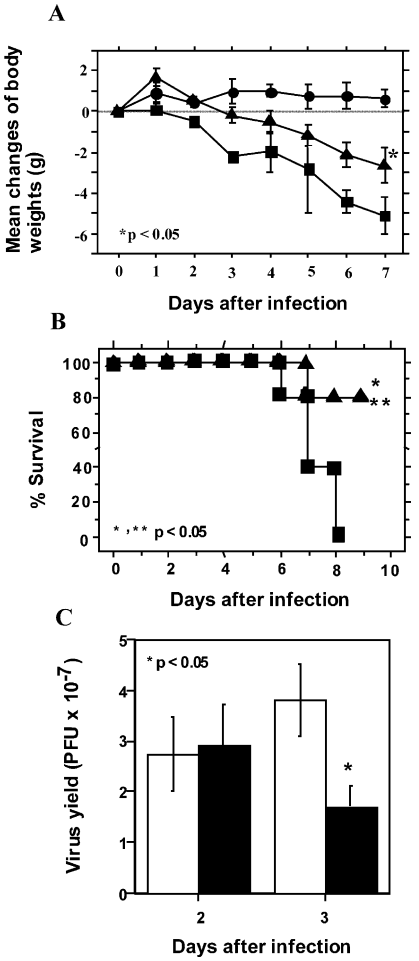

Fig. 1

Fig. 1. (A) Changes in the mean body weights of influenza virus-infected mice. KT (▲) and water (■) were administered to the infected mice (five mice in a group) and the body weights of infected mice were measured daily. ● indicates mock-infected group ($n = 5$). Bars indicate standard errors. $*P < 0.05$, statistical significance compared with the infected mice with water-administration for days 0–7 after infection by the repeated measures of two way analysis of variance followed by the Dunn's procedure as a multiple comparison procedure. (B) Effect of KT on the survival time of influenza virus-infected mice. Mice were intranasally infected with influenza virus. KT (▲) and water (■) were orally administered as described in text. Five mice were used in each group. $*P < 0.05$, statistical significant prolongation of mean day to death compared with the water-administered group by the Kaplan–Meier method for 9 days after infection. $**P < 0.05$, statistical significance compared with the water-administered group by the Fisher's exact test. (C) Effect of KT on virus yields in the BALF of infected mice. Mice were intranasally infected with influenza virus and KT (■) or water (□) was orally administered as described in text. The virus titers in the BALF were determined from six mice in each group on days 2 and 3 after infection. Bars indicate standard errors. An asterisk indicates statistical significance vs. water-administered group on day 3 by the Student's t-test.

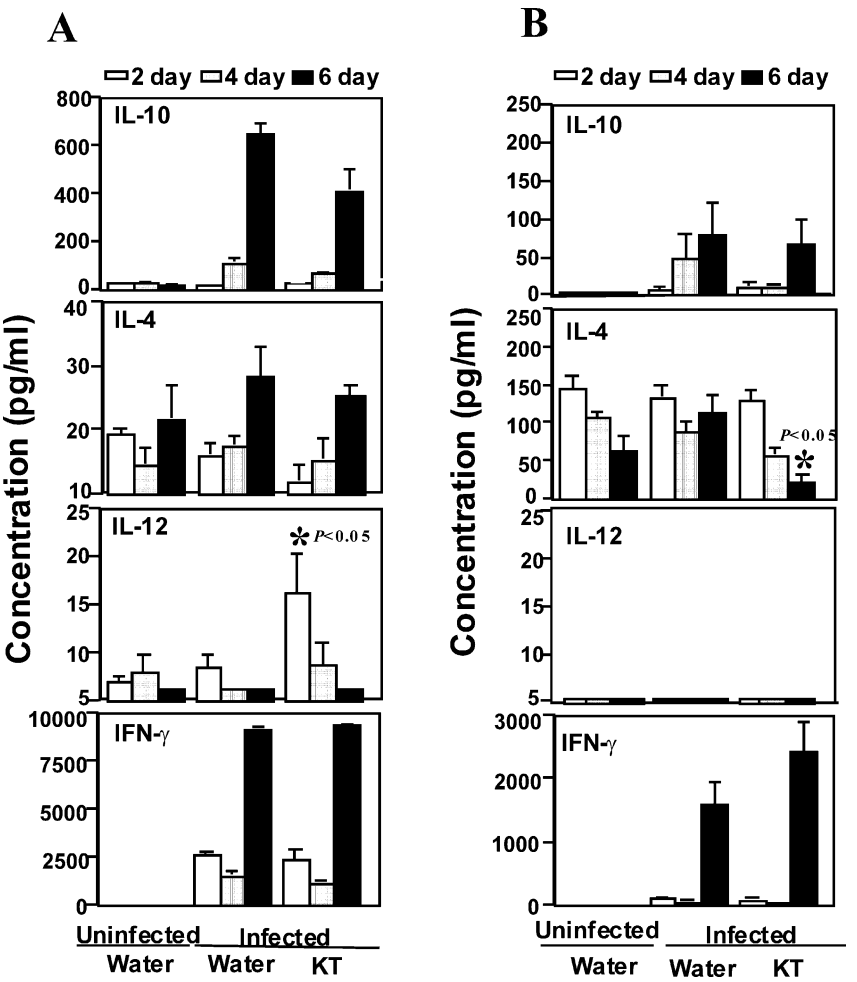

Fig. 2. Changes of cytokine levels in BALF (A) and serum (B) of influenza virus-infected mice administered with KT. KT was administered to the infected mice and BALF and serum were prepared from the mice ($n = 4$ or 6) on days 2, 4 and 6 after infection. The levels of IL-4, -10 and -12 and IFNγ were determined by ELISA. Bars indicate standard errors. Asterisks indicate statistical significance vs. infected mice with water administration on each day by the Student's t-test.

We previously showed that the augmentation of IL-12 production by the administration of clarithromycin and the intranasal administration of IL-12 in the respiratory tract of influenza virus-infected mice reduced virus yield in the BALF and was crucial for the alleviation of influenza infection (Tsurita et al., 2001). In this previous study, IL-12 production was augmented in BALF on day 2 after infection. The significant higher level of IFN-γ, Th1 cytokine, in BALF was detected in the clarithromycin-administered mice only on day 3 and, thereafter, the elevated levels of IFN-γ were similar in mice administered with and without clarithromycin (Tsurita et al., 2001). In this study, the augmentation of IL-12 production by KT was observed in the BALF on day 2 and the increased levels of IFN-γ were similar in mice treated with and without KT on days 4 and 6, which coincided with the previous study. Although we did not measure the IFN-γ levels on day 3, it might be possible that the IFN-γ level on day 3 was also elevated by KT similarly to the case of clarithromycin. This possibility was supported by the facts that virus yields in BALF on day 3 were

significantly reduced by either of clarithromycin or KT. Thus, this study using KT confirmed our previous results with IL-12 augmentation and suggested that KT induced dominant Th1 response in the early phase of infection in mice. The level of IFN-γ was markedly increased in the BALF and serum by influenza virus infection on day 6 (Fig. 2A and B). Th1 immune response may have augmented locally and systemically on day 6. Since IFN-γ suppresses the generation of Th2 immune response the inhibition of growth of Th2 cells (Mosmann and Coffman, 1989), the systemic diminution of level of IL-4 in KT-administered mice on day 6 (Fig. 2B) may be based on Th1 response strengthened by the augmented IL-12 secretion. Thus, KT may contribute the alleviation of infection mainly by the development of Th1 immune response.

IL-12 has been shown to be a factor for controlling the alleviation of influenza infection by using anti-IL-12 antibody (Monteiro et al., 1998). However, the intraperitoneal administration of IL-12 to infected mice daily for days 1–4 after infection was not effective in prolonging the survival times of infected mice (Kostense et al., 1998). In this study, IL-12 production by KT was augmented on day 2 and might be responsible for the significant prolongation of survival times and reduction of mortality. The alleviation of influenza infection in mice is primarily mediated by anti-influenza CD8+cytotoxic lymphocyte activity (Eichelberger et al., 1991; Bender et al., 1992; Kambayashi et al., 2000; Zhang et al., 2000). IFN-γ and cytolytic activities are important as the other factors to lead to the alleviation (Baumgarth and Kelso, 1996; Bender et al., 1994; Price et al., 2000). These factors are based on Th1 immune response. Further, the generation of serum neutralizing antibody is crucial for the alleviation, which is developed by Th2 immune response followed by humoral immune response (Palladino et al., 1995).

KT timely augmented IL-12 production on day 2. The timely augmentation might promote Th1 immune response in contributing the prolongation of survival times. Such increase in IL-12 production in the early phase of influenza was confirmed to influence the survival of infected mice. The augmentation of IL-12 production played a role in the alleviation of influenza infection.

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