Cytokine modulating effect of ginseng treatment in a mouse model of *Pseudomonas aeruginosa* lung infection

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Abstract

The major cause of morbidity and mortality in cystic fibrosis (CF) patients is chronic *Pseudomonas aeruginosa* lung infection. In a mouse model of *P. aeruginosa* lung infection mimicking that in CF patients, the effects of ginseng treatment on cytokine responses and the correlation between the changes in cytokine production and the lung pathology were studied. Mice were challenged with alginate beads containing *P. aeruginosa* (10⁶ CFU/ml). A saline extract of ginseng was injected subcutaneously at a dosage of 250 mg/kg of body weight/day for 7 days. Saline was used as a placebo control. One week after challenge, a significantly lower mortality was found in the ginseng treated group (*P*<0.005). The lung cells from the ginseng treated group produced more interferon-gamma (IFN-γ) (*P*<0.04) and tumor necrosis factor-alpha (TNF-α) (*P*<0.03) but less interleukin-4 (IL-4) (*P*<0.02) with a higher ratio of IFN-γ/IL-4 (*P*<0.004) after 6 and/or 24 h of incubation with specific and non-specific antigens as compared to the control group. The ginseng treated splenocytes produced more TNF-α (*P*<0.03) and IFN-γ (*P*<0.05) than the control spleen cells. Furthermore, a significantly milder lung pathology (*P*<0.025) and a faster bacterial clearance (*P*<0.038) from the lungs were also found in the ginseng treated group compared to the control group. These results indicate a Th1-like immune response in the mice with *P. aeruginosa* lung infection after 7 days of ginseng treatment, which is an important mechanism accounting for ginseng’s favorable action. We therefore believe that Th1 response might benefit the host with *P. aeruginosa* lung infection and ginseng treatment might be a promising alternative measure for the treatment of chronic *P. aeruginosa* lung infection in CF patients.

Keywords: *Pseudomonas aeruginosa*; Ginseng; Cytokine; Animal model; Cystic fibrosis

1. Introduction

Cystic fibrosis (CF) patients are characteristically susceptible to chronic *Pseudomonas aeruginosa* lung infection with increasing ages [1]. The bacteria grow in alginate biofilm and produce β-lactamase to protect themselves from the host immune responses and the action of antibiotics [1−3]. It is, therefore, difficult to eradicate the chronic *P. aeruginosa* lung infection with antibiotic treatment. The recurrent lung infection of *P. aeruginosa* will gradually destroy the lung tissues leading to poor prognosis. Clearly, an alternative and effective solution is needed for the control of the infection. The natural course of chronic *P. aeruginosa* lung infection in CF patients or in rat and mouse models is a Th2 helper type 2 (Th2)-like response [2,4−8], i.e., the pronounced antibody response against *P. aeruginosa* antigens leads to the formation of immune complexes in the lung tissues and in turn the infiltration of polymorphonuclear leukocytes (PMN) resulting in lung tissue damage. We therefore speculate that a Th1 dominated immune response might favor the host with chronic *P. aeruginosa* lung infection.

Previously we have shown that treatment with crude ginseng extract suppressed serum antibody responses against *P. aeruginosa* antigens, decreased IgG1 but increased IgG2a in serum, reduced mast cell number in the lung foci of the rat model with chronic *P. aeruginosa* lung infection [7−10]. At the same time, the lung
pathology became milder and the lung bacterial clearance was greatly enhanced [7–10]. However, the mechanism behind the favorable actions of ginseng treatment is unknown. In the present study, ginseng treatment was conducted to a mouse model of P. aeruginosa lung infection and the therapeutic effects as well as the cytokine dichotomy of Th1/Th2 responses in the infection primed lung cells in the comparison with a control group were studied.

2. Materials and methods

2.1. Animals

CBA/J mice: Eighty-six healthy female CBA/J mice (M&B Laboratory animals, Ry, Denmark), 11-weeks old with body weights of 22–30 g were used. The mice were divided into two groups, 39 mice in the ginseng treated group and 47 in the control group.

2.2. Challenge strain

Mucoid P. aeruginosa PAO 579 (International Antigenic Typing System O: 2/5) [11,12] was used in our study.

2.2.1. Immobilization of P. aeruginosa in seaweed alginate beads

The method was described previously [13] In brief, 1 ml of the P. aeruginosa bacterial culture was mixed with 9 ml of seaweed alginate (60% guluronic acid content) and the mixture was forced once with air through a cannula into a solution of 0.1 M CaCl2 in 0.1 M Tris–HCl buffer (pH 7.0). The suspension was adjusted to yield 10⁶ CFU/ml and the yield was confirmed by colony counts.

2.2.2. Preparation of ginseng extract

The ginseng saline extract was prepared using the method described previously [8] In brief, 2.5 g of Panax ginseng C.A. Meyer (ginseng) powder (provided kindly by Millingwang Limited, Jilin, PR China) was mixed with 100 ml of saline, and then the mixture was heated and filtered through sterile filter paper twice before use (final concentration: 25 mg of the equivalent of dry powder per ml). The concentration of protein in the ginseng extract was 3.5 mg/ml and the level of endotoxin-like material was 60 ng/ml [8] which is 1660 times lower than the amount of lipopolysaccharide (LPS) that we used in another study [14] The two parameters are used as the quality control of the ginseng extract.

2.3. Treatment protocol

The ginseng treated group: 2.5% of ginseng saline extract was injected subcutaneously (250 mg/kg) once a day for 7 days. The dosage was decided according to the results of a pilot study for dosages. The control group: sterile saline (0.9%) was injected subcutaneously (10 ml/kg) once a day for 7 days as a placebo control.

2.4. Challenge procedures

At the time of challenge, all mice were anesthetized by subcutaneous injection of a 1:1 mixture of etomidat (Janssen, Birkerød, Denmark) and midazolam (Roche, Hvidovre, Denmark) at a dose of 10 ml/kg of body weight and tracheotomized [15,16]. Intratracheal challenge with 0.04 ml of P. aeruginosa (10⁵ CFU/ml) in alginate beads was performed with a bead-tipped needle [15,16]. The incision was sutured with silk and healed without any complications. Seven days after challenge, all animals were killed by using 20% pentobarbital (DAK, Copenhagen, Denmark) at 2 ml/kg body weight.

The challenge and the start of administration of ginseng or saline were on the same day.

2.5. Macroscopic pathology of the lungs

The macroscopic lung pathology was expressed as the lung index of macroscopic pathology (LIMP), which was calculated according to the formula: LIMP = the lung area with pathologic changes/the area of the same side lung (left lung) [7,17]. The lung pathological changes include lung abscess, atelectasis, consolidation, hemorrhage, and lung edema. The evaluation was done blindly.

2.6. Histopathologic scoring of the lungs

The cellular alterations in the lungs were classified as acute or chronic inflammation by a scoring system based on the proportion of neutrophils (PMN) and mononuclear leukocytes (MN) in the inflammatory foci [18]. Acute inflammation was defined as an inflammatory infiltration in which PMNs were predominant (>90% PMNs with <10% MNs), whereas chronic inflammation was defined as a preponderance of MNs (>90% with <10% PMNs), which included lymphocytes, plasma cells, and the presence of granulomas [1,18]. Four scoring groups were assigned microscopically to evaluate the severity of the inflammation, as follows [7,8,10,18]: (1) normal histology; (2) mild and focal chronic inflammation; (3) moderate to severe focal chronic inflammation mixed with areas of normal lung tissue; and (4) acute inflammation to necrosis or severe chronic inflammation throughout the lung. The histopathological evaluations were done in a double-blind way to avoid bias.
2.7. Lung bacteriology

The lungs from one-third of the animals in each group were selected randomly for the quantitative bacteriological examination as described previously [15,16]. In brief, lungs were removed aseptically and homogenized in 5 ml of PBS. Appropriately diluted samples were plated on agar plates to determine the number of CFU.

2.8. Preparation of lung and spleen cell suspensions [15,16]

By the time of killing, mouse lungs were exsanguinated by cutting the vena cava inferior and infusing PBS to the right ventricle. The lungs were then washed once in the complete RPMI 1640 medium [with 2% fetal calf serum (FCS), Biological Industries, Kibbutz Beit Haemek, Israel], 10 mM of HEPES (Panum Institute, Denmark), 20 units of penicillin/ml, 20 μg of streptomycin/ml, 100 μg of gentamycin/ml, 58.4 μg of l-glutamine and 0.05 mM of 2-mercaptoethanol]. The lungs were cut into small pieces in 10 ml of the complete RPMI 1640 medium containing 2 mg of collagenase/ml (Sigma Chemical Co., St. Louis, USA) and 50 μg of DNase/ml (Boehringer Mannheim GmbH, Mannheim, Germany). The lung pieces were incubated in shaking condition for 1.5 h at 37°C. Free cell suspension was obtained by repeated pipetting and by passing through a 18-G needle followed by a 23-G needle. The cells were washed 3 times with the complete RPMI 1640 medium and resuspended in 6 ml of the complete RPMI 1640 medium with 10% FCS. The cells were stained with crystal-violet and counted for the cell concentration, which was adjusted to 2×10⁶ cells/ml.

Spleens were obtained aseptically and perfused several times with 10 ml of 10% FCS-complete RPMI 1640 medium. Erythrocytes were removed by 0.17 M of NH₄Cl and washed twice with 10% FCS-complete RPMI 1640 medium (350 g, 10 min) and resuspended in 5 ml of 10% FCS-complete RPMI 1640 medium. Number of the cells were confirmed by counting in trypsin blue (viability >90%) and were adjusted to 4×10⁶ cells/ml.

2.9. Antigens and mitogens

The outer membrane protein (OMP) of P. aeruginosa was prepared by the following method [15,16]: the P. aeruginosa sonicate in distilled water was mixed with 20% of Sarkosyl (100 μl/ml) and centrifuged in 3000×g for 10 min after 30–60 min of staying in room temperature. The supernatant was centrifuged again and washed once with distilled water in 20 000×g for 30 min. The deposition was resuspended in distilled water and filtered by a syringe filter with a pore size of 0.20 μm.

The final OMP concentration in the medium was 10 μg/ml. Concanavalin A (ConA) (Pharmacia Fine Chemicals AB, Uppsala, Sweden) for non-specific stimulation was employed in a final concentration of 4 μg/ml. Monoclonal hamster anti-mouse CD3e without sodium azide (anti-CD3) (Pharmingen, San Diego, USA) for short stimulation of already activated T-cells was used in a final concentration of 0.5 μg/ml. OMP and ConA were incubated with the cells for 24 h and anti-CD3 was incubated with the cells for 6 h. Medium without antigen or mitogen was used as control for 6 or 24 h. The above time points of antigens or mitogen stimulation for the cell culture were decided upon the pilot kinetic studies, in which the time points of 6, 12, 24, 48 and 72 h were tested (data not shown).

2.10. Cytokine production

One hundred microliter of the proper adjusted cell suspensions were added to each well in round bottomed micro-titer plates (Nunc, Roskilde, Denmark) and 100 μl of medium with OMP, ConA, anti-CD3 or medium alone were added to the cells in triplicates [15,16]. Cells were incubated at 37°C in a 5% CO₂. Supernatants from the lung cells stimulated with anti-CD3 were harvested after 6 h of incubation, and the supernatants from the lung cells stimulated with OMP and ConA were harvested after 24 h. The samples were kept at −70°C until use.

The concentrations of interleukin-4 (IL-4), interferongamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) in the supernatants were determined by ELISA kits (Genzyme, Cambridge, USA). IL-4 standard curve range from 62.5 to 1000 pg/ml (sensitivity 10 pg/ml), IFN-γ standard curve range from 20 to 1620 pg/ml (sensitivity 10 pg/ml), and TNF-α standard curve range from 125 to 2000 pg/ml (sensitivity 15 pg/ml).

2.11. Statistical analysis

Unpaired differences in continuous data were analyzed by the Mann–Whitney U test and ANOVA test, and categorical data were compared using the χ²-test. The correlation analysis between the parameters was performed by the Simple Regression.

3. Results

3.1. Mortality

In the ginseng treated group, 5 out of 39 mice died from lung infection (12.8%) whereas 21 out of 47 mice in the control group (44.7%) were dead due to the lung infection. The difference between the two groups was significant, P<0.005. The mortality was determined 7
days after challenge and the death happened mainly 2–3 days after challenge.

3.2. Pathology

3.2.1. Macroscopic pathology of the lungs

One week after P. aeruginosa lung infection, the LIMP in the ginseng treated group (n=34) was 0.27 (range: 0.15–0.67) or 0.30±0.11 (mean±S.D.), significantly lower than that in the control (n=26) group [0.50 (range: 0.27–0.93), or 0.54±0.21], P=0.0001. Simple Regression analysis showed that the LIMP in the ginseng treated group was positively correlated with the lung bacteriology (r=0.78, P<0.02).

3.2.2. Histopathology of the lungs

Nine mice from each group were selected randomly for histopathological examination. The acute inflammatory response was found mainly in the bronchia, and the lung tissues around the bronchia were also involved. The damage of bronchial wall and significant infiltration of inflammatory cells in the bronchia and the lung tissues were seen in both groups (Figs. 1 and 2). However, the inflammatory reaction in the ginseng treated group was less acute (PMNs <50% and MN >50%; Fig. 3) and milder compared to the control group (P<0.025, Table 1).

Table 1

<table>
<thead>
<tr>
<th>Treatment (n)</th>
<th>Inflammation Acute (in bronchi)</th>
<th>Acute-chronic</th>
<th>Score 2⁺</th>
<th>Score 3⁺</th>
<th>Score 4⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ginseng (9)</td>
<td>1 (11%)***</td>
<td>8 (89%)***</td>
<td>5 (56%)**</td>
<td>2 (22%)</td>
<td>2 (22%)*</td>
</tr>
<tr>
<td>Control (9)</td>
<td>7 (78%)</td>
<td>2 (22%)</td>
<td>0</td>
<td>2 (22%)</td>
<td>7 (78%)</td>
</tr>
</tbody>
</table>

Compared with the control group, *P<0.025, **P<0.01, ***P<0.005.

a See Section 2.

b The proportion of PMN:NM = <50%: >50%.
3.2.3. Lung bacteriology

Seven days after intratracheal challenge with alginate beads containing *P. aeruginosa*, 9 mice were selected randomly from each group for the examination of lung bacteriology. The median lung bacterial count in the ginseng treated group was 50 (0–12 750) CFU/lung, and in the control group it was 18 300 (0–1 625 000) CFU/lung, \( P < 0.038 \). The median lung bacterial count in the ginseng treated group was 365 times lower than in the control group.

3.2.4. Cytokine production

Nine and eight mice were selected randomly from the ginseng treated and the control groups, respectively, for the lung and spleen cytokine examinations.

3.2.4.1. IFN-\( \gamma \). After 6 h of incubation with anti-CD3 antibody and 24 h of culture with OMP of *P. aeruginosa*, the production of IFN-\( \gamma \) from the ginseng treated lung cells was significantly higher in the culture supernatant as compared with the control group (\( P < 0.04 \), Table 2). The ratio of IFN-\( \gamma \)/IL-4 after 6 h of incubation with anti-CD3 antibody in the ginseng treated group was higher than that in the control group (\( P < 0.004 \)). In the response to ConA after 24 h, no significant difference was found between the two groups. Higher IFN-\( \gamma \) production was also seen in the ginseng treated spleen cells (Table 3). Regression analysis showed that the lung IFN-\( \gamma \) level in the ginseng treated group was negatively correlated with its IL-4 level after 6 h incubation with anti-CD3 antibody in lung cells (\( r = -0.67, P < 0.008 \)).

3.2.4.2. TNF-\( \alpha \). No significant difference was found in TNF-\( \alpha \) production from the lung cells after 6 h of incubation with anti-CD3 antibody. However, 24 h after culture, TNF-\( \alpha \) production from the ginseng treated lung cells stimulated with ConA (\( P < 0.0002 \)) and OMP (\( P < 0.03 \)) was increased (Table 2). Higher TNF-\( \alpha \) production was also seen in the spleen cell culture from the ginseng treated group (\( P < 0.02 \)) compared to the control group (Table 3).

3.2.4.3. IL-4. The IL-4 production in the lung cells from the ginseng treated group was significantly lower than that from the control group after 6 h of incubation (\( P < 0.0002 \)).

### Table 2

**Cytokine production from the lung cells 7 days after *P. aeruginosa* lung infection in mice [median (range) pg/ml] and [mean \( \pm \) S.D. pg/ml]**

<table>
<thead>
<tr>
<th>(a) Median (range) pg/ml</th>
<th>Antigens (time)</th>
<th>Ginseng (( n = 9 ))</th>
<th>Control (( n = 8 ))</th>
<th>( P ) values</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-( \gamma )</td>
<td>Anti-CD3 (6 h)</td>
<td>120 (40–260)</td>
<td>30 (1–150)</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td></td>
<td>OMP (24 h)</td>
<td>355 (100–815)</td>
<td>265 (30–570)</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td></td>
<td>ConA (24 h)</td>
<td>2420 (1530–3330)</td>
<td>2100 (570–2750)</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>IL-4</td>
<td>Anti-CD3 (6 h)</td>
<td>75 (30–150)</td>
<td>105 (60–210)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>IFN-( \gamma )/IL-4 (6 h)</td>
<td>4.00 (0.44–26.00)</td>
<td>0.45 (0.001–1.38)</td>
<td>&lt;0.004</td>
</tr>
<tr>
<td></td>
<td>ConA (24 h)</td>
<td>115 (30–180)</td>
<td>180 (90–222)</td>
<td>&lt;0.018</td>
</tr>
<tr>
<td>TNF-( \alpha )</td>
<td>Anti-CD3 (6 h)</td>
<td>120 (40–370)</td>
<td>125 (0–190)</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td></td>
<td>OMP (24 h)</td>
<td>970 (400–1955)</td>
<td>310 (125–1130)</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td></td>
<td>ConA (24 h)</td>
<td>1260 (930–1775)</td>
<td>650 (510–890)</td>
<td>&lt;0.0002</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(b) Mean ( \pm ) S.D. pg/ml</th>
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<tbody>
<tr>
<td>IFN-( \gamma )</td>
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<tr>
<td></td>
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<tr>
<td></td>
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<tr>
<td>IL-4</td>
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<td></td>
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<tr>
<td>TNF-( \alpha )</td>
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</tbody>
</table>

Anti-CD3, anti-CD3 antibody; OMP, outer membrane proteins of *P. aeruginosa*; ConA, Concanavalin A.

### Table 3

**Cytokine production from the mouse spleen cells 24 h after incubation with OMP of *P. aeruginosa* [median (range) pg/ml] and [mean \( \pm \) S.D. pg/ml]**

<table>
<thead>
<tr>
<th>(a) Median (range) pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (n)</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Ginseng (9)</td>
</tr>
<tr>
<td>Control (8)</td>
</tr>
<tr>
<td>( P ) value</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(b) Mean ( \pm ) S.D. pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ginseng (9)</td>
</tr>
<tr>
<td>Control (8)</td>
</tr>
<tr>
<td>( P ) value</td>
</tr>
</tbody>
</table>
Ginseng is a well-known Chinese traditional medicine, which has been used in China as a valued herbal remedy for more than 4000 years [19]. Traditionally, ginseng is used as a tonic, rejuvenating and revitalizing agent, whereas currently it has been demonstrated that ginseng possesses a wide range of pharmaceutical and therapeutic effects on many systems such as the central nervous system, cardiovascular system, endocrine system and immune system, etc. [19,20]. However, ginseng’s potential functions of anti-microorganism infection are poorly investigated.

Chronic *P. aeruginosa* lung infection in CF patients is characterized by a pronounced serum antibody response against the pathogen and the infiltration of numerous PMNs in the lung tissues, which have been shown to be connected to a poor prognosis [1,2,21,22] and is a Th2-like immune response [6]. Previously we had shown in a rat model of chronic *P. aeruginosa* lung infection that ginseng treatment down-regulated the excessive antibody response and reduced the severity of lung pathology [7]. Furthermore, mast cell number in the lung foci reduced [8,10] and serum IgG2a increased but IgG1 decreased [7] after ginseng treatment, indicating a Th1-like response.

In the present study, ginseng treatment induced a significantly higher IFN-γ and TNF-α, but lower IL-4 production and higher ratio of IFN-γ/IL-4 in the lung cells from the mice with *P. aeruginosa* lung infection, consistent with a Th1-like response that has been seen in our previous work. In the IFN-γ response, ConA did not induce a higher IFN-γ production in the ginseng treated lung cells whereas OMP did, indicating such IFN-γ response was a specific one. The significantly lower mortality seen in the ginseng treated group suggests a strong protection against *P. aeruginosa* lung infection due to ginseng treatment. Milder lung pathology and a greatly enhanced lung bacterial clearance found in the ginseng treated group 7 days after intratracheal *P. aeruginosa* challenge indicate the powerful modulating effects of ginseng in the bacterial infection. The positive correlation between lung pathology and bacteriology in the ginseng treated group indicates a clear relationship between the pathogen number and the severity of lung pathology.

Host immune response against microbial infection could be divided into two different types according to the activation of T helper cell type and the changes in cytokine patterns. Th1 response is characterized by the production of IFN-γ and IL-2, which favors cellular immunity and results in an increase of serum IgG2a level, whereas Th2 response is associated with the secretion of IL-4 and IL-5 as well as the activation of B lymphocytes and mast cells, enhances the development of humoral immunity and induces the production of IgG1 in serum [23–27]. In our rat model of chronic *P. aeruginosa* lung infection, higher serum IgG and IgG1 response and more mast cells in the lung foci were seen in the placebo control group indicating a Th2-like response compared to the ginseng treated group [7–10]. However, ginseng treatment successfully induced a shift of the immune response from a Th2-like to a Th1-like, which led to a significantly enhanced bacterial clearance and milder lung pathology [7–10]. In the present study, a similar result was found in the mouse model of *P. aeruginosa* lung infection as well, and the cytokine reaction from the lung cells showed a Th1 dominated cytokine profile in the ginseng treated group, which might account for the favorable effects of ginseng on the animals with *P. aeruginosa* lung infection.

IFN-γ is known to inhibit the production of IL-4 and, therefore, down-regulate the antibody response [23,25–27]. The negative correlation between IFN-γ and IL-4 seen in the ginseng treated group after 6 h of incubation in our study is consistent with the conclusion. IFN-γ is produced by T lymphocytes and natural killer (NK) cells [28] and is able to activate the microbicidal function by macrophages and NK cells [28]. Furthermore, IFN-γ can enhance the chemotaxis and phagocytosis of PMNs to the pathogens [29]. Previously we had found that ginseng treatment improved the phagocytosis to *P. aeruginosa* by PMNs, macrophages and lung macrophages in rats [30]. Our results also showed a stronger TNF-α response in the lungs due to ginseng treatment indicating the activation of phagocytes in the lungs. In vitro study also demonstrated that ginseng stimulation increased the TNF-α production from the mouse splenocytes and macrophages [31]. Yang et al. reported that ginseng treatment significantly increased the phagocytosis by macrophages and enhance NK activities in mice [32,33]. An in vitro investigation of ginseng showed that ginseng significantly enhanced NK activity and also the antibody-dependent cell-mediated cytotoxicity (ADCC) in the peripheral blood mononuclear cells from both healthy subjects and patients with chronic fatigue syndrome or AIDS [34]. Similar results were also reported by other authors [35–38]. We speculate that the activated NK cells and ADCC function might also be involved in the mechanism of ginseng’s favorable action observed in our study.

The cytokine results from the ginseng treated spleen cells might suggest a systemic activation of a Th1-like response against *P. aeruginosa* lung infection due to...
ginseng treatment. Our previous results have demonstrated that the outcome of \textit{P. aeruginosa} infection in a Th1 responding animal was much better than in a Th2 responder [15,16], and IFN-\gamma treatment very significantly improved the prognosis of \textit{P. aeruginosa} infected rats \[18,39\]. In CF patients, Th1 response can help to clear the infection and keep better lung functions [6]. The response of IL-4 from the lung cells after 24 h of incubation was unfortunately not detectable using ELISA method in the study. The reason might be due to the consumption of the cells in a longer time of incubation.

In the present study, the favorable therapeutic effects in the ginseng treated group come from the administration of a crude ginseng aqueous extract. It has been known that ginseng contains more than 70 different kinds of components, such as saponins, polysaccharides, proteins, fatty acid, and so on [19]. Yet, it is not clear that which component(s) of ginseng is(are) responsible for the therapeutic effects found in our study. It is important to isolate and characterize the active compounds from ginseng for further study.

In summary, ginseng treatment induced a Th1-like response in the mice with \textit{P. aeruginosa} lung infection, which might activate the phagocytes and NK cells to clear the bacterial infection more effectively, and down-regulate the antibody response which could reduce the formation of immune complexes in lungs and results in less lung tissue damage. We therefore conclude that Th1 response might benefit the host with chronic \textit{P. aeruginosa} lung infection and ginseng treatment might be a promising alternative measure for the treatment of chronic \textit{P. aeruginosa} lung infection in CF patients. To clarify the active compounds in ginseng, further study is needed.

References