Bacterial Translocation Is Reduced by a Specific Nutritional Combination in Mice with Chemotherapy-Induced Neutropenia\textsuperscript{1,2}

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Abstract

Immune function is compromised in many cancer patients, leading to an increased risk of (infectious) complications. Chemotherapy-induced neutropenia is a common cause of treatment-induced immune suppression. In the present study, the effect of a specific nutritional combination (SNC) on bacterial translocation was studied in a model of chemotherapy-induced neutropenia in C3H/HeN mice colonized with \textit{Pseudomonas aeruginosa} PAO-1. Dietary intervention started after stable colonization with \textit{P. aeruginosa} to compare the SNC containing high protein, L-leucine, fish oil, and specific oligosaccharides to an isoenergetic control diet. After 3 wk, the mice were treated with cyclophosphamide to induce neutropenia. This rendered the mice susceptible to \textit{Pseudomonas} translocation, which was quantified 5 d later. Intervention with the SNC resulted in a reduced incidence and intensity of bacterial translocation to the liver (\(P < 0.05\)) and a similar trend in the lungs (\(P \approx 0.057\)). In addition, the SNC reduced the fecal pH (\(P < 0.05\)) and decreased \textit{P. aeruginosa} counts in fecal samples (\(P < 0.05\)). Moreover, plasma concentrations of proinflammatory cytokines were correlated with the reduced bacterial translocation to the liver (\(r > 0.78, P < 0.001\)). In conclusion, dietary intervention with the SNC significantly reduced the incidence and severity of \textit{P. aeruginosa} translocation in a mouse model of chemotherapy-induced immune suppression. Several mechanisms might have played a role, including the modulation of the intestinal microbiota, an improved gut barrier function, immune function, and a reduced inflammatory state. These results suggest an opportunity to develop new applications in cancer patients, with the aim to reduce infectious and other complications. J. Nutr. 141: 1292–1298, 2011.

Introduction

In many cancer patients, immune function is compromised due to host-, tumor- and treatment-related factors, leading to an increased risk of (infectious) complications. In addition, age, stress, depression, and nutritional status are important factors that may contribute to the immune deficiency (1,2). A systemic inflammatory state accounts for the production of chemokines, cytokines, prostaglandins, and reactive oxygen/nitrogen species inducing profound immune suppression, which facilitates the escape of tumor cells from immune surveillance (3–5). Similar inflammatory mediators may be produced by the tumor itself as well, which in turn can facilitate angiogenesis, tumor cell growth, and the recruitment of myeloid-derived suppressor cells (3,5–7). Myeloid-derived suppressor cells are a population of CD11b\textsuperscript{+}/GR-1\textsuperscript{+} cells that contribute to tumor escape and immune suppression and they are a potential link between inflammation and tumor progression. The risk of immune deficiency-induced complications is even higher after cancer treatment. Surgery, radiotherapy, and chemotherapy are associated with suppression of the cellular immune system and lead, in combination with malnutrition, to a reduced treatment efficacy and a higher frequency and severity of (infectious) complications (1,8–11). In addition, cancer treatment can induce a change in patients’ intestinal microbiota and encourage damage of the gastrointestinal (GI)\textsuperscript{7} mucosa leading to severe inflammation and a diminished barrier function (12–15). To reduce the risk of

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\textsuperscript{2} Supplemental Figure 1 and Supplemental Table 1 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.

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\textsuperscript{7} Abbreviations used: C, control diet; C-P, control diet and \textit{Pseudomonas aeruginosa}; C-PT, control diet, \textit{Pseudomonas aeruginosa}, and chemotherapy; C-T, control diet and chemotherapy; GI, gastrointestinal; GOS, galactooligosaccharide; P:S, polyunsaturated:saturated; SNC, specific nutritional combination; SNC-PT, specific nutritional combination diet, \textit{Pseudomonas aeruginosa}, and chemotherapy; SNC-T, specific nutritional combination diet and chemotherapy.
(infectious) complications and to support the performance state of cancer patients, a multi-targeted approach should be applied, including nutritional support. Every effort should be made to prevent involuntary weight loss and delayed treatment schedules. In malnourished patients, preoperative nutritional support is associated with a 50% reduction of postoperative complications (11), but benefits of oral supplementation, including decreased GI toxicity, improved performance status, and increased immune responses, were described as well (16). Therefore, nutritional interventions have been recommended as an integral part of cancer therapy to improve clinical outcomes and quality of life (8,11,16).

In daily practice, chemotherapy often accounts for the development of severe neutropenia, which is defined as an absolute neutrophil count < 500 cells/mm³ and is an important risk factor for the development of bacterial infections (9,17). Infections by Gram-positive as well as Gram-negative bacteria were observed in neutropenic cancer patients, in which coagulase-negative staphylococci and *Staphylococcus aureus* are the most common Gram-positive bacteria. *Escherichia coli*, Klebsiella species, and *Pseudomonas aeruginosa* are the most common Gram-negative pathogens isolated from neutropenic patients and account for 60–65% of documented bacterial infections. Moreover, Gram-negative infections have been associated with a higher morbidity and mortality in advanced disease (18,19). In neutropenic cancer patients, the opportunistic pathogen *P. aeruginosa* can cause severe infections of the respiratory tract that may lead to sepsis. Other mucosal sites, including the GI tract, the urinary tract, and the eyes, can be colonized by *P. aeruginosa* as well. Moreover, the bacterium can easily translocate from the GI tract into the bloodstream as a consequence of chemotherapy-induced GI mucosal damage (20–22).

In previous studies in tumor-bearing mice, a recently developed specific nutritional combination (SNC) containing high protein, L-leucine, fish oil, and specific oligosaccharides was shown to ameliorate tumor-induced suppression of Th1-mediated immunity and reduce the inflammatory state. The complete SNC was necessary for this effect, because no effects were observed with the individual nutritional ingredients (23). Similarly, the SNC, but not the individual ingredients, beneficially affected the tumor-induced catabolic state and preserved muscle mass and function (24). In the current proof of concept study, functional effects of the SNC were studied in an immune-compromised situation in chemotherapy-induced neutropenic mice, as a model for anti-cancer treatment-induced infectious complications. Translocation of a clinically relevant pathogen, *P. aeruginosa*, was used as the main functional outcome in this study.

### Methods

#### Study design

To establish a stable colonization with *P. aeruginosa*, female C3H/HeN mice were pretreated with the broad-spectrum antibiotic ampicillin (i.p. injection, 200 mg/kg dissolved in 0.2 mL saline, Sigma-Aldrich Chemie) for 3 consecutive days (d 0,1, and 0) (Supplemental Fig. 1). The mice in the *P. aeruginosa* groups were infected with 0.2 mL ampicillin-resistant *P. aeruginosa* strain PAO-1 (ATCC BAA-47: 10^12 CFU/L in PBS + 3% bicarbonate) administered by oral gavage (infection on d 0) as previously described (20,25,26). The colonization of the GI tract with *P. aeruginosa* was measured in fecal samples and a stable gut colonization was obtained after 5 d as observed in previous validation experiments. Colonization of the GI tract with *P. aeruginosa* remained stable after antibiotic treatment and restoration of the otherwise normal microbiota. On d 6, the mice in the *P. aeruginosa* groups were randomized on the basis of their colonization level and body weight before the start of the dietary intervention with either control diet or SNC diet. The diets were refreshed every day and the food intake per cage was recorded. During the experiment, weight development was measured 3 times/wk, and several times per week fresh feces samples were obtained from individual mice to follow the colonization of the gut with *P. aeruginosa*.

After 3 wk of dietary intervention, neutropenia and mucosal damage were induced by chemotherapy. On d 28 and 30, the mice in the chemotherapy groups received i.p. 100 mg cyclophosphamide (in sterile saline, Sigma-Aldrich Chemie) per kilogram body weight leading to *P. aeruginosa* dissemination and systemic translocation. The other groups received an i.p. injection with only saline solution. After chemotherapy, blood was drawn daily from different mice in selected experimental groups by submandibular puncture to determine the degree of neutropenia. This was performed in such a way that each of the mice in these groups provided 1 blood sample after chemotherapy. In addition, after chemotherapy, body weight, food intake, and health score of the mice were monitored daily and fresh feces samples were collected to determine colonization levels of *P. aeruginosa*. In contrast to studies described in the literature, the validated model used in this experiment examined only morbidity of the mice rather than mortality.

At d 33, 5 d after the first chemotherapy dose, blood of the mice was collected by cardiac puncture and sampled in heparin tubes to measure proinflammatory cytokines to establish the inflammatory state of the mice and neutropenia was assessed by hematological analysis. After the mice were killed, liver and lungs were removed aseptically to determine translocation of *P. aeruginosa* to these organs (25,27,28). In addition, spleen, brain, thymus, and the skeletal muscles were dissected. Intestinal content samples were obtained by ileum lavage (using 1 mL sterile demi water) and removal of the cecum content. The pH of these samples was measured and they were frozen at −80°C.

#### Mice and diets

Seven to eight-week-old female C3H/HeN mice were obtained from Charles River. All experimental procedures were approved by the Animal Experimental Committee and complied with the principles of laboratory animal care. Mice were housed in groups of 5 in individual ventilated cages to prevent contamination among groups and all handlings were performed in a laminar flow cabinet. The individual ventilated cages were placed in a climate-controlled animal care facility (12-h-light/dark cycle with a constant room temperature of 21°C and humidity of 50%). All mice had free access to food and sterilized drinking water. Upon arrival, mice were acclimatized for 1 wk. During this acclimatization period and before the start of the dietary intervention, the mice received a maintenance diet [AIN-93M (29), Research Diet Services].

The presented results are representative of 2 separate experiments. To confirm the results of the first experiment, it was repeated with a similar setup. In both experiments, mice were divided into different groups receiving either a control diet supplied as pellets or an experimental diet enriched with a SNC containing high protein, L-leucine, fish oil, and a specific mixture of prebiotic oligosaccharides. Both diets were isoenergetic and based on a semisynthetic AIN-93M diet with a modified fat source. In the control diet, the soy oil was replaced with corn oil, which was used as the base oil in the SNC diet as well. The control diet contained per kg food: 125.8 g protein (100% casein), 697.7 g carbohydrates, 32.6 g fat (100% corn oil) with a polyaunsaturated:saturated (P:S) fat ratio of 4.4 and a (n-3):(n-6) fatty acid ratio of 0.014, and 50.0 g fiber (cellulose). The SNC diet contained per kg food: 209.6 g protein (188.6 g intact protein, of which 68% was casein, 32% was whey, and 21% was free L-leucine), 540.3 g carbohydrates, 52.5 g fat (20.2 g corn oil, 10.2 g canola oil, and 22.1 g fish oil containing 6.9 g EPA and 3.1 g DHA with a P:S fat ratio of 4.0 and a (n-3):(n-6) fatty acid ratio of 0.9), 50.0 g fiber of which 22.0 g was cellulose, 19.8 g short-chain galacto-oligosaccharides (GOS; Vivinal GOS, Friesland Domon Foods), 6.0 g lactose from GOS syrup, and 2.2 g short-chain fructooligosaccharides (Beneo p95®,Orrafti) (Supplemental Table 1).

The study focused on the 3 main experimental groups: the group receiving the control diet without any treatment (C; n = 10), the group receiving C diet and *P. aeruginosa* and chemotherapy (C-PT; n = 20), and the group receiving SNC diet and *P. aeruginosa* and chemotherapy.
(SNC-PT; \( n = 20 \)). Because colonization and bacterial translocation of \( P. \) \textit{aeruginosa} could not be measured in group C, this group is not shown in the colonization and translocation graphs (Figs. 2 and 4). Moreover, to control the effects of \( P. \) \textit{aeruginosa} and chemotherapy treatment and to exclude potential interactions between the chemotherapy and the SNC diet, 3 additional reference groups were added to the study (Supplemental Table 1). Results from these groups did not show bacterial translocation or any other unexpected findings and are, for that reason, not mentioned in the paper.

\textbf{P. \textit{aeruginosa}}

\textit{Culture.} \( P. \) \textit{aeruginosa} strain PAO-1 (ATTC BAA-47) was subcultured on Nutrient Agar and inoculated into trypticase soy broth (500 mL). The overnight culture was washed, concentrated, and diluted to a concentration of \( 1 \times 10^{12} \) CFU/L in PBS (D-PBS, Invitrogen) and 3% bicarbonate (for neutralization of gastric acid) as determined by spectrophotometry (OD\textsubscript{600}). The number of bacteria was confirmed by plating dilutions (in PBS) of bacteria on \( P. \) \textit{aeruginosa} C-N selective supplement agar (Oxoid).

\textit{Determination of colonization.} At regular time intervals, fresh feces samples were collected from each mouse by placing the mice individually in a clean and empty cage for 5 min. The samples were weighted, diluted, and homogenized. Subsequently, the pH of the samples was measured and they were diluted in peptone physiological salt solution before plating on C-N agar with 20 mg/L ampicillin. After overnight incubation at 37°C, the number of \( P. \) \textit{aeruginosa} (CFU) per gram feces was determined.

\textit{Bacterial translocation to liver and lungs.} Aseptically removed organs were collected in 0.5 mL buffered peptone water, weighted, and homogenized (4°C) by using an ultra-turrax (IKA, autoclavable disposable turrax). Ten-fold dilution series were prepared in PBS and plated on nutrient agar and C-N agar plates. After overnight incubation at 37°C, the number of specific \( P. \) \textit{aeruginosa} colonies and non-specific colonies was determined.

\textit{Hematology} To determine white blood cell and RBC count and differential, heparinized blood was diluted \( 2 \times \) with sterile physiological salt solution and was measured on the ADVIA120 Hematology system (Siemens).

\textit{PGE\textsubscript{2} and cytokine measurement} Prostaglandin E2 (PGE\textsubscript{2}) in plasma was measured using a commercial anti-PGE\textsubscript{2} rabbit polyclonal antibody-based direct enzyme immunoassay (Oxford Biomedical Research) according to the manufacturer’s protocol. Cytokines in plasma were measured using a commercial mouse cytokine stabilization but remained different (\( P < 0.05 \)). After the chemotherapy treatment, food intake was monitored daily, but no significant differences between the groups were observed.

\textit{P. \textit{aeruginosa} colonization, pH, and beneficial bacteria.} Five days after the \( P. \) \textit{aeruginosa} infection, a stable colonization was obtained, as observed in previous validation experiments (data not shown). On d 6, the mice in the \( P. \) \textit{aeruginosa} groups were randomized on the basis of their colonization level (5.7 \( \pm \) 0.2 log\textsubscript{10} CFU/g feces in the C-PT group vs. 5.6 \( \pm \) 0.2 log\textsubscript{10} CFU/g feces in the SNC-PT group) and body weight. Subsequently, several times per week fresh feces samples were collected from individual mice to follow the colonization of the gut with \( P. \) \textit{aeruginosa} (Fig. 2). At d 13, the colonization of \( P. \) \textit{aeruginosa} in the SNC-PT group was still significantly higher than in the C-PT group (\( P < 0.05 \)). After the chemotherapy treatment, \( P. \) \textit{aeruginosa} levels in fecal samples of the C-PT and SNC-PT groups decreased, but at d 30 and 31 the

\textbf{Results}

\textit{Body weight and food intake.} In the first part of the study, a normal bodyweight curve was observed without any differences among the 3 groups (Fig. 1). From d 20 onwards, the increase in body weight was more pronounced in the SNC-PT group compared with the C and C-PT groups. After the chemotherapy treatment of both the C-PT and SNC-PT groups, body weight declined compared with the C group. However, the body weight in the

\textbf{FIGURE 1} Body weights of mice in the C, C-PT, and SNC-PT groups. Data are mean ± SEM, \( n = 18 \) or 10 (C). *Different from C-PT, \( P < 0.05 \) (least significant difference test).

\textbf{FIGURE 2} \( P. \) \textit{aeruginosa} colonization levels in individual fresh feces samples of the mice in the C-PT and SNC-PT groups. Data are means ± SEM, \( n = 18 \). *Different from C-PT, \( P < 0.05 \) (Mann-Whitney U test).
colonization in the SNC-PT group was still lower than in the C-PT group ($P < 0.05$). In addition, the fecal pH of the mice in the SNC-PT group were lower at d 7 and 31 (pH 7.4 and 8.1, respectively) compared with the fecal pH in the C-PT group (pH 8.1 and 8.5, respectively; $P < 0.05$). Besides the colonization of $P. aeruginosa$, the presence of beneficial bacteria was measured at several time points. At d 28 and 33, more lactobacilli were found in the feces of the mice in the SNC-PT group ($10.9 \pm 0.13$ and $11.1 \pm 0.35 \log_{10} \text{CFU/g}$, respectively; $P < 0.05$) compared with the C-PT group ($10.3 \pm 0.16$ and $10.4 \pm 0.35 \log_{10} \text{CFU/g}$, respectively). Bifidobacteria could hardly be detected in the feces of the mice in the different groups and showed a large variation.

Chemotherapy-induced neutropenia. After the chemotherapy treatment, the mice in the C-PT and SNC-PT group became severely neutropenic compared with the mice in the C group ($P < 0.05$) (Fig. 3A). Moreover, the absolute number of lymphocytes (Fig. 3B) and the total white blood cell count (data not shown) decreased ($P < 0.05$). The numbers of the different cell types did not differ between the C-PT and SNC-PT groups.

$P. aeruginosa$ translocation. At d 33, after the mice were killed, bacterial translocation of $P. aeruginosa$ to liver and lungs was measured. The translocation incidence to the liver was lower in the SNC-PT group compared with the C-PT group ($P < 0.05$) (Fig. 4A), whereas the other experimental groups did not show any translocation. In addition, the incidence of $P. aeruginosa$ translocation to the lung tended to be lower in the SNC-PT group compared with the C-PT group ($P = 0.053$). The mice that died of infection were included in the translocation analysis by giving them the highest score of translocation, because translocation can be expected in these mice. Accordingly, there may have been an effect on survival in the SNC-PT group, because only 1 mouse died due to infection compared with 3 mice in the C-PT group. Besides the translocation incidence, the $P. aeruginosa$ counts in the liver were also lower in the SNC-PT group compared with the C-PT group ($P < 0.05$) (Fig. 4B) and the translocation intensity of lung also tended to be lower ($P = 0.057$).

Plasma cytokines and PGE$_2$. The proinflammatory cytokines IL-1$\beta$, IL-6, TNF$\alpha$, IFN$\gamma$, and the inflammatory mediator PGE$_2$ were measured in plasma after the mice were killed at d 33 (Table 1). All cytokine concentrations were greater in the C-PT group than in the C group ($P < 0.001$) and tended to be lower in the SNC-PT group than in the C-PT group ($P = 0.10–0.38$). High within-group variability likely resulted in the lack of significant differences. However, these proinflammatory cytokines were correlated with bacterial translocation intensity to the liver ($r = 0.78–0.82; P < 0.001$).

Discussion

The present study demonstrates a significant reduction of bacterial translocation after dietary intervention with the SNC in a...
mouse model for chemotherapy-induced neutropenia. Previous studies demonstrated that the complete SNC, but not the individual ingredients, induced beneficial immune modulatory effects in tumor-bearing mice (23), suggesting that the SNC might support the resistance to infections in a compromised host. In the current proof of concept study, functional effects of the SNC were investigated in an immune-compromised mouse model of chemotherapy-induced neutropenia as a clinically relevant model for anti-cancer treatment-induced infectious complications, in which the translocation of *P. aeruginosa* was measured as the primary outcome variable. Several mechanisms might have played a role in the effects observed in this study, including modulation of the intestinal microbiota, beneficial effects on gut barrier function, immune function, and a reduced inflammatory state.

As expected, chemotherapy treatment induced a strong reduction in the number of neutrophils, lymphocytes (Fig. 3), and, consequently, total white blood cells (data not shown). However, no differences were observed after the intervention with the SNC on either neutrophil or lymphocyte count. In general, chemotherapy-induced neutropenia and lymphopenia result in suppression of the innate immune system as well as the adaptive immune system, resulting in a high risk of bacterial infections (30). However, the chemotherapy-induced elimination of neutrophils and lymphocytes is not the only risk factor for bacterial translocation. The fact that the SNC significantly reduced the translocation of *P. aeruginosa* to liver and lungs can also be explained by a beneficial effect on the integrity of the intestinal mucosa by decreasing the number of colonized *P. aeruginosa* in the intestine (Fig. 2) or by improving immune cell function rather than the number of immune cells, comparable with results observed in previous experimental mouse models (23). Although this study focused on functional outcomes rather than mechanistic evidence, the current findings and previous data provide suggestions about the mechanisms involved. In the present study, mice were treated with a short course of antibiotics to enable the GI colonization with *P. aeruginosa* that remained stable after the subsequent restoration of the normal GI microbiota. Therefore, modulation of the metabolism or composition of the microbiota may have played a role in the observed effects, especially because the specific oligosaccharides (GOS, fructooligosaccharides) are present in the SNC. These nondigestible oligosaccharides are fermentable fibers with probiotic properties that have been associated with immune modulatory effects and other health benefits, including an improved gut barrier function (31). Although the present study provides no data on the intestinal integrity, it did show a significant reduction of fecal pH after dietary intervention with the SNC. This might be the result of intestinal SCFA production, including butyrate, acetate, and lactate, formed by the fermentation of the prebiotic oligosaccharides by the colonic microbiota, which can contribute to a restoration of the intestinal barrier function (31–33). Accordingly, the reduced pH in the intestinal lumen can lead to the inhibition of pathogen growth and adhesion. Furthermore, the fermentation of the specific oligosaccharides can stimulate the growth of beneficial bacteria as bifidobacteria and lactobacilli, which in turn can also inhibit pathogens by the production of antimicrobial substances (31,34).

The percentage of these bacteria tended to be higher at multiple time points in the group receiving the SNC diet compared with the groups receiving the C diet (33). At d 33, significantly more lactobacilli were detected, which might have contributed to the reduced levels of *P. aeruginosa* infection in the GI tract. Because intestinal colonization of *P. aeruginosa* is a predictor of systemic infections (28,35), the observed reduction in colonization might contribute to the beneficial effect of the SNC on *P. aeruginosa* translocation. Consequently, in severely immune-compromised subjects, a reduction in bacterial translocation to extra-intestinal sites might similarly lead to a reduction of septic morbidity (28).

Another explanation for the beneficial reduction of *P. aeruginosa* translocation by the SNC is its potential effect on the reduced immune function of the host (27). As mentioned before, the specific oligosaccharides can modulate the immune system via a microbiota-dependent (prebiotic) mechanism, but they may also directly affect immune function by blocking or activating specific receptors on immune cells, leading to improved immune responses and eventually to enhanced resistance to systemic infections. Moreover, the specific oligosaccharides might reduce the binding of *P. aeruginosa* on specific receptors leading to a decreased capability to infect (31). Nevertheless, in previous studies in tumor-bearing mice, it was demonstrated that only the complex mixture of high protein, 1-leucine, fish oil, and specific oligosaccharides was able to enhance Th1-mediated immunity and to reduce the inflammatory state (23). In the present study, the beneficial effects might be due to the combination of fish oil and oligosaccharides affecting the above-mentioned risk factors as well, because fish oil is also associated with decreased levels of bacterial translocation and a reduced systemic inflammatory state (36–39). Fish oil contains high amounts of the (n-3) long-chain PUFA EPA and DHA, which play a major role in the regulation of immune responses and inflammation (40–42). In the present study, the severe inflammatory state of the mice after chemotherapy-induced bacterial translocation was confirmed by the increase of the proinflammatory cytokines IL-1β, IL-6, TNFα, and IFNγ in plasma. In addition, the proinflammatory cytokines and the bacterial translocation intensity to the liver were correlated (*P < 0.001*), which we consider a marker for systemic infection due to bacterial translocation from the GI tract. After dietary intervention with the SNC, all the cytokines tended to decrease compared with the C-PT group (*P = 0.097–0.38*). This might be the result of both the antiinflammatory effect of fish oil and the immune modulatory and prebiotic effect of the specific oligosaccharides.

### TABLE 1 Plasma concentrations of proinflammatory cytokines and PGE₂ in mice in the C, C-PT, and SNC-PT groups

<table>
<thead>
<tr>
<th></th>
<th>IL-1β</th>
<th>IL-6</th>
<th>TNFα</th>
<th>IFNγ</th>
<th>PGE₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>10</td>
<td>15</td>
<td>0.1-0.1</td>
<td>2.8-2.8</td>
<td>1.4-1.4</td>
</tr>
<tr>
<td>C-PT</td>
<td>12</td>
<td>21.7</td>
<td>6.0-86.2</td>
<td>1830-891-8030</td>
<td>56.4-43.0-252</td>
</tr>
<tr>
<td>SNC-PT</td>
<td>17</td>
<td>6.0</td>
<td>1.5-42.0</td>
<td>912-43.0-5540</td>
<td>10.0-2.8-114</td>
</tr>
</tbody>
</table>

Correlation with translocation to liver

|   | ρ = 0.82 | ρ = 0.82 | ρ = 0.78 | ρ = 0.80 | ρ = 0.26 |

1 Data are median (IQR) or Spearman correlation coefficient. *Different from C-PT, P < 0.001 (Kruskal-Wallis test); †P < 0.001.
The SNC, containing high levels of protein and the BCAA L-leucine, is hypothesized to modulate metabolism, because it beneficially affected the tumor-induced catabolic state, preserved muscle mass and function, and significantly reduced loss of body weight in previous experiments (24). In the present study, body weights were higher in mice fed the SNC diet compared with mice fed the C diet from d 20 onwards, although food intake was comparable between groups. Therefore, this effect is attributed to differences in diet compositions and the associated modulation of the metabolism. In addition, after chemotherapy treatment, body weight in the SNC-PT group was significantly higher than in the C-PT group, which was associated with a lower loss of muscle and fat mass (data not shown) and could be due to a reduced inflammatory state and/or the high levels of protein and L-leucine in the SNC.

In this and previous studies, the choice was made to investigate the effects of (n-3) PUFA enrichment, the addition of the oligosaccharides and high levels of protein and L-leucine in a test diet with a normal macronutrient composition for mice, based on the AIN-93M definition (43). Because the envisaged application of the SNC concept in humans is an oral nutritional supplement aimed at increasing total protein intake and increasing the relative (n-3) PUFA intake, the test and C diets were designed to be isoenergetic but not isonitrogenous. Because the total dietary intake of the mice was not expected to differ between groups, this setup allowed us to test a relative increase in protein and (n-3) PUFA intake. To study a good contrast between the test and C diets with regard to fatty acid composition, the base fat source of the AIN-93M-based diets was modified, replacing soy with corn oil. Corn oil, containing a (n-3):(n-6) fatty acid ratio of 0.014, was used as a base fat source that is highly predominant in (n-6) PUFA, similar to Westernized types of diet, although more emphasized. Other parameters of the fat source, such as the total amount of fat and the P:S fat ratio, were kept similar to the normal mouse diet. All aspects of the fat content of a diet play a role in the functional effects of dietary PUFA interventions (44,45), but the choice was made to focus on a change in the (n-3):(n-6) PUFA intake ratio. The relatively high levels of (n-6) PUFA in the C diet might be argued to induce inflammation (40). However, in mice fed the C diet, levels of proinflammatory cytokines were very low compared with the mice in the C-PT group (Table 1), showing no spontaneous induction of inflammation. Moreover, in previous studies in tumor-bearing mice, inflammation did not differ between the use of low levels of soy oil compared with corn oil (23). In the present proof of concept study, a model without the presence of a tumor was used, because it would complicate the model further, making it difficult to control well. For applications in human cancer patients, it is important to gain insight into the relation among the tumor, the chemotherapy treatment, and the nutritional ingredients for safety reasons, to explore the potential benefits, and to optimize the nutritional intervention with regard to dose, composition, schedule of administration, and target populations (44,46).

This study focused on functional outcome parameters after nutritional intervention with the SNC in a mouse model featuring a clinically relevant pathogen. Therefore, the mechanistic evidence is limited in this study and no direct parameters of intestinal inflammation, intestinal permeability, or intestinal immune cell depletion were measured. Future studies using histological techniques and functional analysis of intestinal permeability could provide more insight into the mechanisms that underlie the observed effects. The current model, using a relatively low dose of chemotherapy, was set up to study morbidity, rather than mortality, in a sensitive way. Therefore, this model is thought to be most relevant for the prevention of septic infections rather than for treating and ameliorating the course of existing septic infections. In this sensitive model, we regarded translocation of \( \text{P. aeruginosa} \), combined with elevated and highly correlated levels of serum inflammatory cytokines, as a marker for infection (25,27,28). Other clinical symptoms of infections (e.g., fever, illness score) were not analyzed in these mice. However, because translocation is a necessary step in sepsis with pathogens of intestinal origin, it is argued that the protective effect on translocation is of potential clinical relevance. Although the induction of \( \text{P. aeruginosa} \) colonization does not correspond to the normal clinical exposure to the pathogen, the functional situation after colonization is clinically relevant. In this regard, the model resembles the clinical situation in humans suffering from a quiescent intestinal infection that, e.g., after chemotherapy, surgery, or transplantation, leads to bacterial translocation and results in a severe sepsis (28).

In conclusion, dietary intervention with the SNC significantly reduced the incidence and severity of \( \text{P. aeruginosa} \) translocation in chemotherapy-induced neutropenic mice by reducing the translocation to liver and a similar trend in the lungs. In addition, the SNC reduced the fecal pH, which may at least partly explain the lower \( \text{P. aeruginosa} \) counts in fecal samples during the nutritional intervention phase of the experiment. Plasma levels of proinflammatory cytokines tended to be reduced and a strong correlation was observed with bacterial translocation to the liver. In accordance to the presence of high protein, L-leucine, fish oil, and specific oligosaccharides in the SNC, the underlying mechanism may involve a prebiotic as well as an immune modulatory effect. Together with previous results showing beneficial effects on cellular immunity, muscle function, and body composition in tumor-bearing mice, these results might represent a new opportunity for applications in cancer patients to reduce (infectious) complications.

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