Enhancement of Host Resistance to Microbial Infections in Mice Fed a High Fat Diet by *Lactobacillus casei* Cells

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**ABSTRACT**

The carbon clearance ability of female ddY mice was markedly reduced by feeding with a high fat-low carbohydrate (HFLC) diet for 8 weeks. The reduced clearance ability was restored by a single intraperitoneal (ip) injection with heat-killed *Lactobacillus casei* (YIT 9018) cells (LC 9018). The ip injection of LC 9018 into the HFLC diet-fed mice led to a remarkable enhancement of nonspecific resistance when they were exposed to 50% lethal doses of *Pseudomonas aeruginosa* or herpes simplex virus, type 1. The resistance-enhancing capacity of LC 9018 against *P. aeruginosa* infection was significantly maintained for 7 days after the ip inoculation with LC 9018 in mice fed the HFLC diet. The reduced *in vivo* killing activity of peritoneal exudate cells (macrophage-rich) from mice fed the HFLC diet to *P. aeruginosa* infection was markedly augmented by the ip administration of LC 9018. The results indicate that LC 9018, one of the bacterial immunostimulants, may restore the reduced host defense functions in mice induced by feeding with a high fat diet for a long period.

**Key words:** *Lactobacillus casei*, High fat diet, Opportunistic infection, Immunosuppressed mouse

In the relation between nutrition and the immunological function in mammals, it is known that feeding with dietary fat for a long period causes dysfunctions of the reticuloendothelial immune systems, and as a result increases the incidence of several cancers\(^1\),\(^4\),\(^7\) and rheumatoid arthritis\(^13\). For instance, polyunsaturated fatty acids and polyunsaturated fatty acids (n-6) may suppress cellular immunity\(^10\), and polyunsaturated fatty acids (n-3) may also cause the accumulation of lipid peroxidative products and reactive oxygen species during the inflammatory process\(^6\).

We have reported that the host resistance to infection with *P. aeruginosa* and herpes simplex virus in mice fed a high fat diet was markedly reduced, in comparison with animals fed a high protein diet, and that the reduced host resistance was restored by the addition of vitamin E to the high fat diet\(^10\).

Moreover, we have found that heat-killed *L. casei* (LC 9018) markedly enhanced the host resistance to several microbial infections in normal and immunosuppressed animals\(^17\),\(^18\),\(^20\),\(^21\), and that LC 9018 induced various immunobiological changes including the activation of macrophages\(^17\),\(^20\), the activation of natural killer (NK) cells\(^8\), and the activation of antibody-producing cells\(^14\),\(^22\) in addition to the antitumor capacity\(^9\),\(^22\).

The aim of this study was to obtain new information about the effect of bacterial immunostimulants on animals in which immunodeficiency has been induced by feeding with a high fat diet for a long term. The present study focused on the resistance-enhancing capacity of LC 9018 to *P. aeruginosa* infection in mice fed a high fat diet.

**MATERIALS AND METHODS**

**Animals and diets**

Four-week-old female ddY mice purchased from Shizuoka Agricultural Cooperative for Experimental Animals (Shizuoka, Japan) were fed with a high fat-low carbohydrate (HFLC) and a standard (ST) diet (as normal controls) for 8 to 11 weeks, respectively. The compositions of the two diets are summarized in Table 1.

**Bacterial immunostimulant**

LC 9018 (a lyophilized preparation of heat-killed *L. casei*, YIT 9018) was donated by Yakult Central Institute for Microbiological Research, Tokyo.
Challenge pathogens
The wild type of pyocyanine-positive *P. aeruginosa* was isolated from a female patient with pyelitis in Mie University Hospital, Mie, Japan. *P. aeruginosa* cells grown in brain-heart infusion (BHI) broth (Eiken Chemicals Co., Tokyo) at 37°C for 18 hr were passed through ddY mice 3 times to enhance the pathogenesis. *P. aeruginosa* cells grown in BHI broth at 37°C for 18 hr were washed 3 times with saline and then suspended in saline. The colony-forming units (CFUs) were enumerated with nalidixic acid-cetrimide (NAC) agar plates (Eiken Chemicals Co.). BHK 21 cells cultured in Eagle's minimal essential medium (MEM; Wako Pure Chemicals Co., Osaka) supplemented with 10% fetal bovine serum (FBS; MA Bioproducts, Walkersville, U.S.A.) were infected by herpes simplex virus, type 1, HF strain (HSV-1). The cultures were subjected to three freeze-thaw cycles and centrifuged at 10,000 rpm for 60 min. The supernatant fluids harvested were frozen at −80°C until use. The plaque-forming units (PFUs) were assayed by the methylcellulose-overlay method with the monolayered BHK 21 cells. Fifty percent of lethal doses (LD<sub>50</sub>) of the two pathogens to normal mice fed the ST diet were used throughout the experiments.

Cytogram and biological analysis of peritoneal exudate cells (PECs) from mice fed the HFLC diet by LC 9018

Mice fed the HFLC diet for 8 weeks (n=3 in each group) were inoculated ip with LC 9018 (0.5 mg/mouse) 3 days before the harvest of PECs. The PECs suspended in 5 ml of Eagle's MEM with 10% FBS (1.0 × 10<sup>5</sup> cells/ml) were incubated on a plastic plate (50-mm) at 37°C for 30 min. The cultured cells were incubated with heat-killed *Staphylococcus aureus* (cell: bacteria = 1 : 10; M.O.I. = 10) at 37°C for an additional 30 min, washed and stained with Giemsa solution. The bacteria-phagocytizing PECs were counted and the cell-populations were observed.

Effect of LC 9018 on carbon clearance ability (CCA) of mice fed the HFLC diet

HFLC diet-fed mice (n=3) were injected via the tail vein with 10 µl of 8 mg/ml carbon particle-suspension from India ink 3 days after the ip inoculation of LC 9018 (0.5 mg/mouse). At various intervals after the injection, the mice were bled from the retro-orbital plex, taking 25 µl of blood. The blood was diluted in 2 ml of distilled water, and then the absorbance was measured at 710 nm. As controls, the blood was bled immediately after the injection of carbon particle-suspension (at zero time). The carbon clearance ability (CCA) was calculated as follows:

The CCA (%) = OD at 0 time – OD at each time/optical density (OD) at 0 time × 100.

Protective action of LC 9018 against *P. aeruginosa* or HSV-1 infection in mice fed the HFLC diet

Mice fed the HFLC diet for 8 weeks (n=10) were challenged ip with either *P. aeruginosa* (4 × 10<sup>6</sup> CFUs/mouse) or HSV-1 (2 × 10<sup>5</sup> PFUs/mouse) 3 days after a single ip administration of LC 9018 (0.5 mg/mouse). The mice fed the ST diet (n=10) were used as a normal control group. The survival rates were recorded on the 7th (to *P. aeruginosa* infection) or the 14th (to HSV-1 infection) day after infections.

Persistence of protective action of LC 9018 on *P. aeruginosa* infection in mice fed the HFLC diet

Mice fed the HFLC diet for 8 weeks (n=10) were inoculated once ip with or without LC 9018 (0.5 mg/mouse) 21, 14, 10, 7 or 3 days before the ip challenge with *P. aeruginosa* (4 × 10<sup>6</sup> CFUs/mouse). During the inoculation period (for 3 weeks), the mice were further fed with the HFLC diet. The survival rates were recorded on day 7 after the challenge.

Assay for *in vivo* killing activity of PECs from mice fed the HFLC diet against *P. aeruginosa* infection

This assay system has been described previously. In brief, mice fed the HFLC diet for 8 weeks (n=3) were challenged ip with *P. aeruginosa* (1.0 × 10<sup>5</sup> CFUs/mouse) 7 days after the ip administration of LC 9018 (0.5 mg/mouse). Three hours later, 2.5 ml of Hanks balanced salt solution (HBSS) containing 4 units heparin/ml was injected into the peritoneal cavity, and then the peritoneal fluid was harvested after gently massaging the abdomen. This procedure was repeated once. In the controls (at zero time), *P. aeruginosa* (1.0 × 10<sup>5</sup> CFUs/mouse) was injected.

Table 1. Compositions of diets

<table>
<thead>
<tr>
<th>Content(%)</th>
<th>Standard (ST)</th>
<th>High Fat-Low Carbohydrate (HFLC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean oil</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>Liver oil</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Dextrin</td>
<td>60</td>
<td>42</td>
</tr>
<tr>
<td>Casein</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Yeast</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Fiber</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Salts*</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

into the peritoneal cavity. The peritoneal fluid was harvested immediately and centrifuged at 150 \( \times \) g for 10 min. The cell-pellets were disrupted with 5 ml of 0.85% \( \text{NH}_4\text{Cl} \) solution for 20 min to release the phagocytosed bacteria from the PECs. Each sample was serially diluted 10-fold with HBSS, and the number of CFUs in the disrupted cells and in the supernatant fluid was determined by plating on NAC agar plates, respectively. The killing activity of the PECs was calculated as follows:

\[
\text{Killing activity} (\%) = \frac{\text{Total CFUs at } 0 \text{ time} - \text{Total CFUs after } 3 \text{ hr/Total CFUs (in disrupted cells + in supernatant fluid)} \text{ at } 0 \text{ time}}{100}
\]

Statistics
Significance between the experimental and control groups was determined by Student’s \( t \)-test. A difference of \( p < 0.05 \) was considered significant.

RESULTS

Comparison of cytogram and phagocytic function of PECs from the HFLC diet-fed mice inoculated ip with or without LC 9018
As shown in Table 2, the total number of cells (PECs) and the percentages of macrophages (M\( \phi \)s), polymorphonuclear cells (PMNs) and lymphocytes in the PECs from mice fed the HFLC diet showed almost the same levels as the normal control mice fed ST diet. However, the percentage of bacteria-phagocytizing cells in the PECs from mice fed the HFLC diet (26.1 \( \pm \) 3.2%) was lower than in the normal control mice (48.8 \( \pm \) 1.9%), that is, the phagocytizing capacity of the PECs was reduced by feeding with a HFLC diet for 8 weeks. On the other hand, the total number of PECs and the percentage of phagocytizing cells in the PECs from mice fed the HFLC diet were markedly augmented by the ip administration of LC 9018 (0.5 mg/mouse) 3 days before this experiment.

Effect of LC 9018 on the CCA of mice fed the HFLC diet.
As shown in Fig. 1, the CCA of normal control mice fed the ST diet increased linearly in the course of time, whereas the CCA of mice fed the HFLC diet was very low, and no elevation was observed during 50 min after the intravenous injection with carbon particles. In contrast, the reduced CCA of mice fed the HFLC diet was effectively restored by the ip administration of LC 9018 (0.5 mg/mouse). However, the restored level of CCA by ip administration of LC 9018 did not attain the level of the CCA of mice fed the ST diet.

Protective action of LC 9018 against \( P. \) aeruginosa and HSV-1 infections in mice fed the HFLC diet
When normal control mice fed the ST diet were challenged ip with either \( P. \) aeruginosa (4 \( \times \) \( 10^6 \) CFUs/mouse = LD\( 50 \)) or HSV-1 (2 \( \times \) \( 10^6 \) PFUs/mouse = LD\( 50 \)), the survival rates were 40 and 60%, respectively. In contrast, the resistance to these pathogens in normal animals was markedly reduced when they were fed with a HFLC diet for 8 weeks, that is, the survival rates decreased to 10 to 20%. However, this survival rate was increased to the same level or higher as that of the normal control animals by the ip administration of LC 9018 (0.5 mg/mouse) 3 days before the challenge (Table 3).

Persistent action of LC 9018 on \( P. \) aeruginosa infection in mice fed the HFLC diet
Table 4 shows the persistence of the protective action of LC 9018 on \( P. \) aeruginosa infection in mice fed the HFLC diet. The reduced survival rates of mice fed the HFLC diet for 11 weeks were elevated by the ip administration of LC 9018 (0.5 mg/mouse), and the survival percentages were maintained for 10 days as compared with those of normal control mice fed the ST diet. However, all the mice fed the HFLC diet died.
Effect of LC 9018 on carbon clearance ability of mice fed the HFLC diet

Mice fed the HFLC diet for 8 weeks (n=3) were injected via the vein with 10 µl of carbon particle-suspension (8 mg/ml) 3 days after the ip injection with or without LC 9018 (0.5 mg/mouse). At indicated intervals after the injection with carbon particles-suspension, mice were bled from the retro-orbital plex. The blood (25 µl) was diluted and the absorbance was measured at 710 nm. The mean carbon clearance ability (%) was determined as described in “Materials and Methods”.

**Table 3.** Protective action of LC 9018 to *P. aeruginosa* and HSV-1 infections in mice fed the HFLC diet

<table>
<thead>
<tr>
<th>Group</th>
<th>Survivors (%)</th>
<th>Survivors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. aeruginosa</em></td>
<td>HSV-1</td>
</tr>
<tr>
<td>ST diet</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>HFLC diet alone</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>HFLC diet + LC 9018</td>
<td>40*</td>
<td>70*</td>
</tr>
</tbody>
</table>

Mice fed the HFLC diet (n=10) were challenged ip with either *P. aeruginosa* (4 × 10⁶ CFUs) or HSV-1 (2 × 10⁶ PFUs) 3 days after the ip injection with or without LC 9018 (0.5 mg/mouse). Survival rates were recorded on the 7th (to *P. aeruginosa* infection) or the 14th (to HSV-1 infection) day after these infections. Mice fed the ST diet (n=10) were used as normal controls. *p < 0.05 between the HFLC diet alone and the HFLC diet + LC 9018.

**Table 4.** Persistence of protective action of LC 9018 on *P. aeruginosa* infection in mice fed the HFLC diet

<table>
<thead>
<tr>
<th>Group</th>
<th>Survivors (%)</th>
<th>Survivors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days after administration of LC 9018</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>ST diet (normal)</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>HFLC diet alone</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>HFLC diet + LC 9018</td>
<td>50*</td>
<td>50*</td>
</tr>
</tbody>
</table>

Mice which fed the HFLC diet for 8 weeks (n=10) were inoculated once ip with or without LC 9018 (0.5 mg/mouse) 21, 14, 10, 7 or 3 days before the ip challenge with *P. aeruginosa* (4 × 10⁶ CFUs). During inoculation period for 3 weeks, mice were fed with the HFLC diet. Mice fed the ST diet (n=10) were used as normal controls. *p < 0.05 between the HFLC diet alone and the HFLC diet + LC 9018.

DISCUSSION

There have been many studies on the effect of dietary fat on the endocrinical and immunological functions in experimental animals. Previously, we have reported that the host resistance to opportu-
The effects of bacterial immunostimulants on the
not occur in animals fed a high protein diet 19).

However, there have been no reports concerning

nistic microbial infections in animals was dra­
tically reduced by feeding with a high fat diet for
a long period, whereas such a phenomenon did
not occur in animals fed a high protein diet 19).

However, there have been no reports concerning

the effects of bacterial immunostimulants on the
correlation between host resistance to infection
and immunobiological functions in animals fed a
high fat diet for a long term.

In the present study, we examined the protective
effects of LC 9018, one of the bacterial im-
munostimulants, on P. aeruginosa and HSV-1
infections (LD50 to normal control mice fed the
ST diet) in mice fed the HFLC diet for 8 weeks
and the immunobiological effects of LC 9018 on
several functions of Mφs in these animals.

The number of PECs and the percentage of
peritoneal Mφs in mice fed the HFLC diet were
almost the same as those in normal control mice
fed the ST diet, whereas the phagocytizing and the in vivo killing abilities of peritoneal PECs
(mainly Mφs) were markedly reduced by feeding
with a HFLC diet. This decrease in the phagocy-
tizing and killing abilities of the PECs may be
due to dysfunctions of Mφs, that is, the HFLC
diet seems to trigger qualitative rather than
quantitative changes in the peritoneal Mφs. The
high fat diet may thus influence multiple rela-
tionships among fatty acids, free radicals, reac-
tive oxygen species and lipid mediators in
inflammatory cells such as Mφs and PMNs, and
may also influence the lipid compositions of bi-
ological changes of these cells 11). It is well known
that the activity of inflammatory cells is responsi-
ble for the appearance of lipid peroxidation prod-

ucts 6,10). When LC 9018 which is known to
activate various functions of Mφs in immunosup-
pressed animals 17,21,22) was applied to mice fed
the HFLC diet, the reduced phagocytizing and
killing abilities of the peritoneal Mφs were mark-
edly restored.

In earlier studies, it has been indicated that
cellular immunity is not important for protection
against P. aeruginosa infection because the main
effectors cells against P. aeruginosa are PMNs and
their functions are enhanced in the presence of
specific immunoglobulin 5,15). On the other hand,
some authors have demonstrated that the protec-
tive mechanism against P. aeruginosa infection is
dependent on cellular immunity, proposing in
particular that Mφs play a critical role in the con-

Fig. 2. In vivo killing activity of the PECs from
mice fed the HFLC diet by ip inoculation with LC
9018
Mice fed the HFLC diet for 8 weeks (n=3) were
challenged ip with P. aeruginosa (1.0 \times 10^5 CFUs)
7 days after the ip administration of LC 9018 (0.5
mg/mouse). Three hours later, the number of CFUs
in the peritoneal cells and in the supernatant fluids
respectively was assayed. The killing activity of
the PECs was determined as described in "Materials
and Methods". Normal control mice fed the ST diet
(■), mice fed the HFLC diet alone (□), LC
9018-administered mice fed the HFLC diet (△).
*p < 0.05 between the PECs from normal control
mice and the PECs from LC 9018-administered
mice fed the HFLC diet.

M0s

PECs

Killing activity(%)
REFERENCES


