# Immunological Benefits of *Enterococcus faecalis* 2001 in Healthy Volunteers

■

Kohei Takahashi[1,2)]　Osamu Nakagawasai[2)]　Masashi Uwabu[3)]
Masahiro Iwasa[4)]　Hiroyuki Iwasa[4)]　Minoru Tsuji[1)]
Hiroshi Takeda[5)]　Takeshi Tadano[2,6)]

**ABSTRACT**

***Background*** Our previous studies showed that *Enterococcus faecalis* 2001（EF-2001）has various effects such as improvement of the intestinal environment and immune system, and antidementia- and antidepressant-like effects in mice.

***Methods*** In the present study, we conducted hematological and immunological tests to evaluate the safety and immunological benefit of EF-2001 in healthy people（age 54.3±5.6 y, six male and four female）.

***Results*** After the subjects were administered EF-2001 for 4 weeks, the alanine aminotransferase level was decreased, while serum IgM was increased. There were no other changes in clinical values between before and after treatment.

***Conclusions*** These results suggest that EF-2001 may safely enhance immunological capacity in normal subjects.

（**Jpn Pharmacol Ther 2021；49：913-6**）

**KEY WORDS**　EF-2001, Immunoglobulin, Open clinical test

## INTRODUCTION

Functional foods containing lactic acid bacteria have been reported to provide health benefits, such as the regulation of intestinal function, and studies have been conducted to evaluate their various effects. Lactic acid bacteria are classified as probiotics, prebiotics, and biogenics. Probiotics are well known to improve the intestinal environment by adhering to intestinal flora,[1)] and have been reported as favorable candidates for the treatment and prevention of disease such as atopic dermatitis through regulation of the host immune system.[2)] However, probiotics need to be ingested in large doses since most are killed by gastric acid.[3)] Heat-killed probiotic bacteria have been used to minimize the effects of acids.[4)] Heat-killed *Enterococcus faecalis* 2001（EF-2001）is a lactic acid bacterium classified as a biogenic that is used as a biological response modifier; it improves the intestinal environment and has been used for a variety of beneficial purposes.[5,6)] Some studies have shown that heat-killed EF-2001 can improve the immune system and exert antitumor activity both in a mouse model[7,8)] and *ex vivo*,[9)] and is very safe for use in either male or female mice.[10)]

[1)] Department of Pharmacology, School of Pharmacy, International University of Health and Welfare　[2)]Division of Pharmacology, Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University　[3)]Ginza Uwabu Medical Clinic　[4)]Nihon Berm Co. Ltd.　[5)]Department of Pharmacology, School of Pharmacy at Fukuoka, International University of Health and Welfare　[6)]Complementary and Alternative Medicine Clinical Research and Development, Graduate School of Medicine Sciences, Kanazawa University

**Table 1　Changes of biochemical parameters after EF-2001 intake**

| | Pre（$n$=10） | Post（$n$=10） | $P$-value |
|---|---|---|---|
| AST（U/L） | 29.8±20.6 | 26.4±13.8 | 0.22 |
| ALT（U/L） | 29.6±23.9 | 22.6±17.2* | 0.040 |
| γ-GTP（U/L） | 49.3±73.8 | 45.2±61.2 | 0.34 |
| BUN（mg/dL） | 15.0±3.4 | 15.1±2.8 | 0.87 |
| Cr（mg/dL） | 0.78±0.22 | 0.79±0.21 | 0.81 |
| WBC（/μL） | 6040±2059 | 5880±1879 | 0.54 |
| RBC（×$10^4$/μL） | 445±54.9 | 448±45.1 | 0.66 |
| Hb（g/dL） | 13.2±2.3 | 13.3±2.2 | 0.58 |
| Ht（%） | 41.5±6.1 | 41.6±6.3 | 0.88 |
| MCV（fL） | 93.1±7.6 | 92.4±8.3 | 0.10 |
| MCH（pg） | 29.6±3.2 | 29.3±2.9 | 0.37 |
| MCHC（%） | 31.7±1.4 | 31.9±1.0 | 0.41 |
| Pt（×$10^4$/μL） | 23.9±8.5 | 25.2±8.0 | 0.077 |

Mean±SD
*$P$<0.05 vs. pretreatment group

These studies suggest that EF-2001 can safely confer an immunological benefit in preclinical tests, while it remains unknown whether EF-2001 has these effects in humans.

Therefore, we conducted hematological and immunological tests to evaluate the safety and immunological benefit of EF-2001 in normal subjects.

## MATERIALS AND METHODS

### 1　Participants and ethical approval

This study was an open-label trial. Ethical approval for this trial was provided by the Ginza Uwabu Medical Clinic Clinical Research Ethics Board（Tokyo, Japan; approval number: 20001）. The trial was conducted in compliance with the Declaration of Helsinki, Ethical Guidelines for Clinical Research, Japan. All the participants received oral and written explanations about the details of this study before signing agreement forms.

Ten healthy volunteers（age 54.3±5.6 y, six male and four female）were enrolled. None of the subjects regularly used an immune-enhancement agent or habitually consumed foods or supplements containing lactobacillus. They were instructed not to change their dietary habits except to stop alcohol consumption during this study.

### 2　Test samples

Commercially available heat-treated EF-2001（BeRM KAIN）was originally isolated from healthy human feces. It was supplied as a heat-killed, dried powder by Nihon BRM Co.（Tokyo, Japan）. Each research participant took EF-2001（1.2 g）orally once a day for 4 weeks before bed.

### 3　Measurement items

Blood samples were collected before the study and on the last day of the study, and used in hematological and immunoserological tests. Tests were performed to quantify alanine aminotransferase（ALT）, aspartate aminotransferase（AST）, gamma-glutamyl transpeptidase（γ-GTP）, blood urea nitrogen（BUN）, creatinine（Cr）, white blood cell（WBC）, red blood cell（RBC）, hemoglobin（Hb）, hematocrit（Ht）, mean corpuscular volume（MCV）, mean cell hemoglobin（MCH）, mean corpuscular hemoglobin concentration（MCHC）, platelet（Pt）, immunoglobulin A（IgA）, and immunoglobulin M（IgM）.

### 4　Statistical analysis

Results are expressed as the mean±standard deviation（SD）. The statistical significance of differences was determined by the paired Student's $t$-test for two-group comparisons. The criterion of significance was set at $P$<0.05. Data analysis was performed using GraphPad Prism 7（GraphPad Software, San Diego, CA, USA）. No adjustment for multiplicity issues regarding endpoints and base points was performed in those statistical analyses.

## RESULTS

### 1　EF-2001 has safety in healthy human subjects

EF-2001 significantly decreased serum ALT between before and after 4 weeks of treatments in healthy human subjects, while there were no changes in the other clinical values（**Table 1**）.

### 2　EF-2001 increases serum IgM in healthy human subjects

EF-2001 significantly increased serum IgM, but not IgA, in normal subjects（**Fig. 1**）.

## DISCUSSION

Clinically, heat-killed EF-2001 has been used previously to improve the enteric environment. Since EF-2001 does not affect body weight, food consumption, hematological tests, blood biochemistry, or urinalysis, EF-2001 is considered to be highly safe in mice,[10] while it is unknown whether EF-2001 affects human health. In the present study, EF-2001 decreased serum

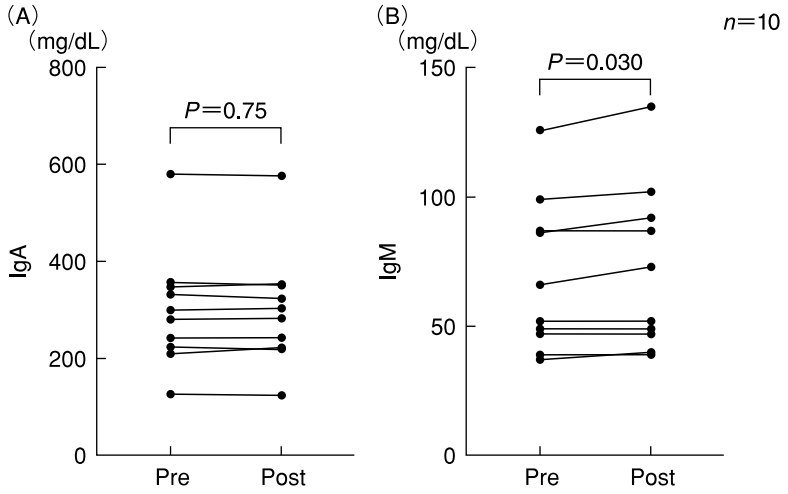


**Fig. 1　Changes in immunoglobulin levels in blood by EF-2001 intake**

ALT between before and after 4 weeks of treatments in healthy human subjects, while there were no changes in the other clinical values. While this change suggests an improvement of hepatic function, we are concerned about the potential that abstention from alcohol drinking during the study period may have also contributed, because another EF-2001 formulation（mouth care BRM-A）did not affect hepatic or kidney function in normal subjects（**Appendix 1**）. Hence, further clinical experiments are needed to examine the effect of EF-2001 on hepatic function. Taken together, our findings suggest that EF-2001 does not worsen the results of hematological tests or blood biochemistry, suggesting that EF-2001 is safe in normal subjects.

In a preclinical study, EF-2001 was shown to have both immunostimulatory and immunoregulatory activities, but no study has assessed the effect of EF-2001 in healthy people. We found that EF-2001 increased serum IgM, but not IgA, in normal subjects［**Fig. 1(B)**］. IgA is classified as serum IgA and secretory IgA. Saliva contains a high proportion of secretory IgA. Interestingly, EF-2001 improved oral candida in patients.[11] Thus, EF-2001 may have an immunological benefit by increasing serum IgM in normal subjects. As IgM is the first antibody produced during an immune response and plays a crucial role in front-line host defense against an antigen,[12] our results suggest that EF-2001 may enhance immune function by increasing serum IgM production, and this effect may be associated with the prevention and control of infections.

## CONCLUSION

EF-2001 does not worsen hematological findings, or hepatic or kidney function, and increases the serum IgM level in normal subjects. Thus, we suggest that EF-2001 may safely enhance immunological capacity in normal subjects.

**【Conflicts of interest】** Masahiro Iwasa and Hiroyuki Iwasa are employees of Nihon Berm Co., Ltd. All other authors declare that they have no competing interests.

**【Acknowledgments】** The authors would like to thank Ms. Miyuki Ikeo of Medical Care Co., Ltd.（Imizu, Japan）, Ms. Masako Aisaka of Kaneroku Pharma Co., Lp（Tokyo, Japan）, and Mr. Kazuhiro Yoshinaga of Sunus Co., Ltd. for experimental advice.

## REFERENCES

1）Fujiwara S, Seto Y, Kimura A, Hashiba H. Establishment of orally-administered Lactobacillus gasseri SBT2055SR in the gastrointestinal tract of humans and its influence on intestinal microflora and metabolism. J Appl Microbiol 2001; 90（3）: 343-52.
2）Kim NY, Ji GE. Effects of probiotics on the prevention of atopic dermatitis. Korean J Pediatr 2012; 55(6): 193-201.
3）Mitsuoka T. Establishment of intestinal bacteriology. Biosci Microbiota Food Health 2014; 33（3）: 99-116.
4）Habil N, Abate W, Beal J, Foey AD. Heat-killed probiotic bacteria differentially regulate colonic epithelial cell production of human β-defensin-2: dependence on inflammatory cytokines. Benef Microbes 2014; 5（4）: 483-95.

5）Takahashi K, Nakagawasai O, Nemoto W, Odaira T, Sakuma W, Onogi H, et al. Effect of Enterococcus faecalis 2001 on colitis and depressive-like behavior in dextran sulfate sodium-treated mice: involvement of the brain-gut axis. J Neuroinflammation 2019; 16（1）: 201.
6）Takahashi K, Kurokawa K, Miyagawa K, Mochida-Saito A, Nemoto Y, Iwasa H, et al. Antidementia effects of Enterococcus faecalis 2001 are associated with enhancement of hippocampal neurogenesis via the ERK-CREB-BDNF pathway in olfactory bulbectomized mice. Physiol Behav 2020; 223: 112997.
7）Choi EJ, Iwasa M, Han KI, Kim WJ, Tang Y, Han WC, et al. Effect of Enterococcus faecalis EF-2001 on experimentally induced atopic eczema in mice. Food Sci Biotechnol 2016; 25（4）: 1087-93.
8）Gu YH, Choi H, Yamashita T, Kang KM, Iwasa M, Lee MJ, et al. Pharmaceutical production of anti-tumor and immune-potentiating *Enterococcus faecalis*-2001 beta-glucans: enhanced activity of macrophage and lymphocytes in tumor-implanted mice. Curr Pharm Biotechnol 2017; 18（8）: 653-61.
9）Tadano T, Tsuzuki S, Nemoto Y, Nakagawasai O, Tan-No K, Onogi H, et al. The role of supplement EF-2001 containing *Enterococcus faecalis* on murine ileal immune responses *ex Vivo*. Pharmacometrics 2017; 93（5/6）: 103-7.
10）Gu YH, Yamasita T, Kang KM. Subchronic oral dose toxicity study of *Enterococcus faecalis* 2001（EF 2001）in mice. Toxicol Res 2018; 34（1）: 55-63.
11）Yamazaki T, Ushikoshi-Nakayama R, Shirone K, Abe M, Matsumoto N, Inoue H, et al. Evaluation of the effect of a heat-killed lactic acid bacterium, *Enterococcus faecalis* 2001, on oral candidiasis. Beneficial Microbes 2019; 10（6）: 661-9.
12）Murphy K, Travers P, Walport M, Janeway C. Janeway's immunobiology. New York: Garland Science; 2008.

Received 24 April 2021; Accepted 27 May 2021

**Appendix 1　Biochemical parameters after EF-2001（mouth care BRM-A）intake**

| | Pre（$n$=9） | Post（$n$=9） | $P$-value |
|---|---|---|---|
| AST（U/L） | 27.7±11.8 | 26.4±13.6 | 0.48 |
| ALT（U/L） | 36.7±33.7 | 41.3±38.6 | 0.43 |
| $\gamma$-GTP（U/L） | 42.7±35.8 | 37.0±26.4 | 0.33 |
| BUN（mg/dL） | 12.3±2.3 | 12.8±2.7 | 0.54 |
| Cr（mg/dL） | 0.78±0.25 | 0.71±0.20 | 0.059 |
| WBC（/$\mu$L） | 6611±1779 | 6144±2302 | 0.19 |

Mean±SD

*　　*　　*