13th INTERNATIONAL CONGRESS of Radiation Research

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Program Guide

Host Organization
Radiation Research Society

In association with the:
SAN FRANCISCO RADIATION ONCOLOGY CONFERENCE
July 7 - 8, 2007
Dear Yeunhwa Gu,

Thank you for submitting your abstract, "Radioprotection effect and anti-tumor immunity by enterococcus faecalis 2001 in mice" to the 13th International Congress of Radiation Research to be held in San Francisco July 8-12, 2007. Your abstract has been accepted for a presentation. Details on presentation information, the size and other instructions on the poster layout will follow.

Meanwhile, if you have not already done so, we suggest you register for the Congress and book your hotel room. You can do this at our web site http://www.icrr2007.org. Note that the period for early registration ends April 13, 2007.

Sincerely,
Martin Brown
Secretary-General

Elizabeth Travis
Chair, Program Committee

13th International Congress of Radiation Research

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Radioprotection effect and anti-tumor immunity by *Enterococcus Faecalis* 2001 in mice

Yeun-hwa Gu\(^a\), Takashi Nakamura\(^a\), Yasuyuki Takagi\(^a\), Yuka Itogawa\(^a\), Hroyuki Iwasa\(^c\), Masahiro Iwasa\(^c\), Takeo Hasegawa\(^a\), Ikukatsu Suzuki\(^a\), Torao Ishida\(^b\) and Masami Oshima\(^a\)

Graduate School of Health Science, Suzuka University of Medical Science, 1001-1 Kishiokacho, Suzuka, Mie 510-0293, Japan\(^a\)
Hi-tec Research Center, Suzuka University of Medical Science, 1001-1 Kishiokacho, Suzuka, Mie 510-0293, Japan\(^b\)
Nihon BMR Research Center, Akasaka Tokyo Blding 9F, 2-14-3, Nagata-cho, Tsioda-ku, Tokyo 100-0014, Japan\(^c\)

Abstract

*Enterococcus Faecalis* 2001 (EF 2001) was good lactic acid bacterium in the human bowels and used the bacillus heat-treated. In the examination of immunoenhancement activity, we measured lymphocyte versus polymorphonuclear leukocyte ratios (L/P activity). The number of lymphocytes was significantly increased in groups treated with EF 2001. The reason that the immature immune response of neonatal mice was activated to the same level observed in mature mice might be because various constituents in the samples used in this experiment activated the neonatal immune system. IFN-γ is a multi-functional factor which shows antiviral effects, suppression of cell growth, anti-tumor effects, activation of macrophages, enhancement of NK cell activation, regulation of immuneresponse, and regulation of the induction of differentiation. Specifically, high levels of IFN-γ were measured in mice bearing the SCC-7, after administration of EF 2001. This strongly suggests that cellular immunity is especially activated by treatment with EF 2001, because production of IFN-γ is limited to the T cells and NK cells stimulated by mitogen and sensitized antigen. EF 2001 showed strong anti-tumor activity against carcinoma. Taken altogether, this strongly suggests that EF 2001 enhances original functions of macrophages and NK cells, and as a result, secondarily enhances the immune reaction and suppresses tumor growth. On the other hand, significant increases in TNF-α suggest an enhancement of T cell growth and induction of cytotoxic activity towards tumor cells and a suppression of tumor growth. Thus, high anti-tumor activity would be expressed more effectively due to direct activity towards tumor cells and activation of a host-mediated immunoreaction.
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Graduate School of Health Science, Suzuka University of Medical Science, 1001-1 Kishiokacho, Suzuka, Mie 510-0293, Japan

Hi-tec Research Center, Suzuka University of Medical Science, 1001-1 Kishiokacho, Suzuka, Mie 510-0293, Japan

Nihon BRM Research Center, Akasaka Tokyo Blding 9F, 2-14-3, Nagata-cho, Tsioda-ku, Tokyo 100-0014, Japan
Material & Methods

• Anti-cancer effects
  ✷ Seven-weeks-old male ICR (Crj) mice
• Cancer cells: sarcoma 180 (2 \times 10^6)
• EF 2001 5mg/kg of heat-killed EF2001 (EF2001) were injected interpretational(endoceliac) each for 2 weeks every other day
  ✷ Statistical methods: t-test
Enterococcus Faecalis under microscope (left) × 20, (right) × 12900
Materials and Methods

1. Radiation protection effects

Seven-weeks-old male C3H mice
12mg/Kg 24mg/Kg of heat-killed EF2001 (EF2001) were injected interpretationally each for 2 weeks every other day

8Gy of whole body irradiation (Philips co. 200kV)

Change of body weight Survival after irradiation

Sections of the large and small intestines with a microscope
2. Assay of NK cell activity by $^{51}$Cr label YAC-1 cells

Injection of the EF2001, in the same condition using the examination of radiation protection effect.

The mice was scarified and the spleen was extracted.

The spleen was smashed with stainless steel mesh, and then mixed PBS and the suspect ion was centrifuged three times.

The concentration of the spleen cells was adjusted to be $2 \times 10^7$ cells/ ml. Then, $1.25 \times 10^6$, $2.5 \times 10^6$, $5.0 \times 10^6$, $10.0 \times 10^6$, $20.0 \times 10^6$ of the spleen cells were added to $1 \times 10^4$ of YAC-1 cell which labeled $^{51}$Cr of 1mCi and incubated 96 hole plate for 6 hours.

Only liquid component in each hole measured with liquid scintillation.
Survival after irradiation
Surviving fraction was increased after injection of EF2001.
Small intestines

control

8Gy

8Gy + 12mg/kg

8Gy + 24mg/kg
NK cells activity
Activities of NK cells are enhanced 1.46 and 1.94 times in EF200112mg and EF200124mg groups respectively.
Anti-tumor effect of BRM on Meth A fibrosarcoma (solid type) in BALB/c mice

*p < 0.05 vs Control*
Time course of increase of lymphocyte/polymorph ratio in mice, a) $P<0.05$, b)
Anti-tumor effect of EF2001 on Salcoma in mice. *P<0.05, **P<0.01
Anti-tumor effect of EF-2001 on S-180 in ICR mice

**P<0.01 *P<0.05 vs Control group
Blood levels of TNF-α in male mice. (pg/ml). There was significant difference in blood levels of TNF-α between the control group and the each group by Dunnett’s test (**p<0.01, *p<0.05).
Fig. 2. C3H mice of IgM in the blood. Each histogram represents the mean value ±SE for 10 mice IgM (M). Significantly different *p<0.05 Control vs. EF 2001.
Fig. 3. C3H mice of IgG in the blood. Each histogram represents the mean value ± SE for 10 mice IgG (M). Significantly different *p<0.05 Control vs. EF 2001.
Conclusion 1

- Anti-cancer effects: *EF 2001* administration group: positive
- *EF 2001* to the radiation protection effect: precision
- Immune activity effect: *EF 2001* administration group: positive
- It put the *EF2001* dosage, and the level of TNF-α in serum glutamic-oxaloacetic increase.
- The level of total IgM in serum glutamic-oxaloacetic increased the *EF2001* dosage group. However, the level of total IgG in serum glutamic-oxaloacetic rather fell slightly.
Conclusion 2

1. It let cell-mediated immunity such as a macrophage and natural killer T cell activate, and the immunization activation action that EF2001 has does not become it, and promotion of humeral immunity anti-action is thought about.

2. In the dosage of EF2001, an IL-2 level in blood rises, and it think that cytokine of the spleen changed from Th2 type into Th1 type.
Antitumor effect

Tumor growth inhibition was accepted by a \textit{EF2001} treated group in comparison with control group, and increase of TNF-\(\alpha\) was accepted.

It is thought that the \(\beta\)-D-glucan which is an active principle of \textit{EF2001}, immunization enhancement and antitumor effect by heteroglucan was obtained by self-cure indirectly.

In addition, it is thought that triterpenoid having loud antitumor effect of \textit{EF2001} acts on a indirect tumor, and immunization enhancement was obtained.