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O-Ring Test

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乳酸産生菌 *Enterococcus faecalis* EF-2001 加熱死菌体の Biological Response Modifier の作用について

柳澤昊永 OM.D.[1)]，岩佐敏廣[1)]， 広部千恵子 Ph.D.[2)]， 下津浦康裕 M.D.,F.I.C.A.E.[3)]

1）日本ベルム BRM 研究所，東京　2）清泉女子大学 文化史学科 教授，東京
3）ORT 生命科学研究所，福岡県

《はじめに》Biological Response Modifier（BRM）は免疫賦活とのかかわりにおいて研究されている生体応答調節物質である。
乳酸産生菌 *Enterococcus faecalis* の加熱死菌体及び菌体成分は，BRM として好中球やマクロファージなどの免疫細胞を活性化する。この *Enterococcus faecalis* に属する EF-2001 株の加熱死菌体を用いた製剤 ORT E-cocci（錠剤），BeRM KAIN，Bio AMB，BeRM Bian（以上ソフト顆粒）はバイディジタル O－リングテストにより適当であることが承認され市場に提供されている。
ここでは，*Enterococcus faecalis* EF-2001 株 加熱死菌体（EFH-201）の外来大腸菌排除促進作用，抗腫瘍効果，鎮痛作用の動物実験結果について報告する。
《結果》
1）EFH-201 の外来大腸菌排除促進作用（感染防御効果）
(a) EFH-201 を飼料に 5%，10%混合して連日与えたマウスに，ヒト由来のストレプトマイシン（SM）耐性大腸菌(3.5×10^9 cfu)を経口投与しマウス腸管からの消失を調べた。SM 耐性大腸菌の消失は，10%混合群で顕著に減少した。
対照群の糞便には多い菌数でしかも遅くまで投与大腸菌が残っていた（Table 1）。
(b) EFH-201 混合飼料を 14 日間与えたマウスに，サイクロフォスファミド（CY）5mg／マウスを腹腔内投与した免疫抑制状態のマウスに，大腸菌の生菌(1.0×10^7 cfu)を静脈内投与し，肝臓内の大腸菌の菌数を調べた。10% EFH-201 で大腸菌数は 48 時間以後有意に低下した。
2）EFH-201 水溶性抽出物の抗腫瘍効果
マウス腹部皮下に腫瘍細胞 Sarcoma-180 を移植後，抽出物の 1mg／マウス量を 3 日間投与し 26 日間飼育した。増殖抑制効果は 70.6%であった。 腫瘍の完全に退縮したマウスも認められた（Fig 1）。
3）EFH-201 水溶性抽出物の鎮痛作用
アスピリンアルミニウムを陽性対照薬として検討した。
鎮痛効果はアスピリンアルミニウム 500mg／kg 経口投与で、51.9%，水溶性抽出物 1000mg／kg の経口投与では 75.8%であった（Table 2）。

【連絡先】〒185-0014 東京都国分寺市東恋ヶ窪 2-29-8　日本ベルム(株)BRM
柳澤昊永 OM.D.　<Tel 042-359-8311；Fax 042-359-8355>

Reactions as Biological Response Modifier of Heat-killed *Enterococcus faecalis* cells

Takaharu Yanagisawa, OM.D.[1], Toshihiro Iwasa[1], Chieko Hirobe, Ph.D.[2], Yasuhiro Shimotuura, M.D.,F.I.C.A.E.[3]

[1] BRM Laboratory of Nihon Berumu Co., Ltd [2] Prof. Department of Cultural History, Seisen University, Tokyo [3] O-Ring Test Life Science Research Institute, Kurume, Fukuoka

《Purpose》 The term " biological response modifier (BRM) means the materials which generally enhance the immune competence in a living body. Heat killed cells or cellular components of *Enterococcus faecalis* among lactic acid producing bacteria, activate immunocytes such as neutrophils and macrophages because they have had BRM functions. The heat killed *E. faecalis* EF-2001 cells [EFH-201] were developed and applied by the bidigital O-ring test to ORT E-cocci (tablets), BeRM KAIN, Bio AMB and BeRM Bian (granules), and they have been supplied as healthy foods in markets. This presentation reports the effects of EFH-201 on elimination of exogenous *Escherichia coli* challenged, and of the water-soluble extract from EFH-201 on anti-tumors and analgesic in experimental animals.

《Results》

1) Exclusive effects of EFH-201 on exogenous *E. coli* challenged.

(a) Mice were given EFH-201 mixed diet for a month, and then challenged orally at a single dose(3.5×10^9 cfu) of streptomycin-resistant *E. coli*. The numbers of the *E. coli* in the feces were significantly reduced in 10% EFH-201 mice after oral administration, and thereafter decreased dose-dependently (table 1). (b) Mice fed EFH-201 mixed diet for 2 weeks were injected with 5 mg of cyclophosphamide. The mice were then infected with an intravenous injection of *E. coli* (1.0×10^7 cfu). The viable numbers of *E. coli* in the liver were decreased significantly of 10% EFH-201 mice 48 and 72 hr after the injection. Therefore, EFH-201 had protective effects against exogenous *E. coli* infections.

2) Anti-tumor effects of the water-soluble extract from EFH-201.

EFH-201 extract, when it was given intratumorally (1mg/mouse/day×3 times), showed anti-tumor effects on Sarcoma 180 and Meth A fibrosarcoma (solid type) transplanted subcutaneously into the abdormens of mice (Fig. 1). The inhibitory ratios of tumor weights by the extract were 70.6 and 56.4% respectively, on day 26 after tumor transplantation.

3) Analgesic effect of the water-soluble extract from EFH-201.

1000mg/kg of EFH-201 given orally showed an analgesic effect on writhing induced by stimulation of diluted-acetic acid administered intraperitoneally into mice (table 2). An analgesic efficacy of aspirin aluminium (500mg/kg) used as a positive control was 51.9% compared with vehicle, while that of EFH-201(1000mg/kg) was 75.8%.

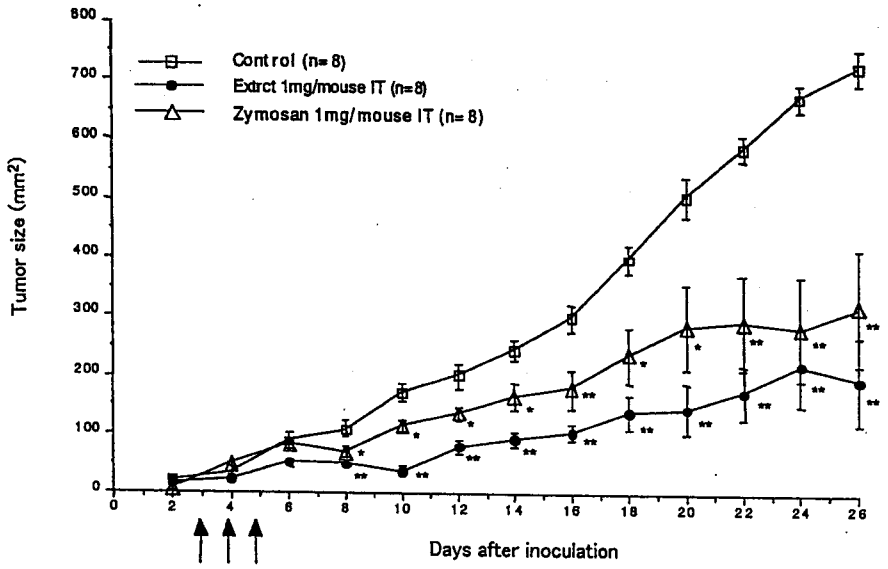

Fig. 1 Antitumor effect of EFH-201 extract on enlargement of Sarcoma-180 in mice.
Sarcoma-180 (1.7×10^6 cells) was inoculated subcutaneusly into the abdormens of mice (n=8) on day 0, and then 1 mg of the extract or zymozan injected intratumorally days 3, 4 and 5 after tumor inoculation (shown by arrows in this figure). The tumor size was measured at indicated intervals.
* P<0.05 and ** P< 0.01 (: Significantly different from control.)

Table 1 Exclusion of SM-resistent *E. coli* challenged orally from feces of mice given EFH-201(viable counts : log cfu/g feces)

Group	Day(s) after challenge of SM-resistent *E. coli*				
	1	2	3	4	7
Control	7.5±0.2	6.3±0.3	5.3±0.2	4.8±0.4 (n=5)	4.6±0.2 (n=3)
5% EFH-201	7.4±0.3	6.1±0.4	4.9±0.4	4.5±0.5 (n=3)	4.2 (n=2)
10% EFH-201	7.2±0.3	4.8±0.2 **	4.2±0.5 *	4.3 (n=2)	3.2 (n=1)

Mice were given 10%EFH-201 diet for a month, and then challenged orally with SM-resistent *E.coli* (3.5×10^9 cfu). Numbers of the E. coli were examined at intervals in feces of mice. Data were expressed as means± standerd error (SE) for 6 mice. *,** Significantly different from control at P<0.05(*) or P<0.01(**). (n=) : number of mice detected for SM-resistent *E. coli* in the feces.

Table 2 Eeffect of water soluble EFH-201 extract on writhing induced by acetic acid in mice

Group	Dose (mg/kg)	No. of writhing (for 10 min)	Inhibition of writhing (%)
Control	0	33.0±4.0	—
EFH-201 extract	1000	8.0±3.8 **	75.8
Aspirin aluminium	500	15.9±4.6 *	51.9

Mice were injected with diluted acetic acid one hour after oral administration of the EFH-201 extract or of aspirin aluminium. Then minutes later, the number of writhing mice were counted for 10 minutes in each mouse.
* P<0.05 and ** P<0.01 (: Significantly different from control.)

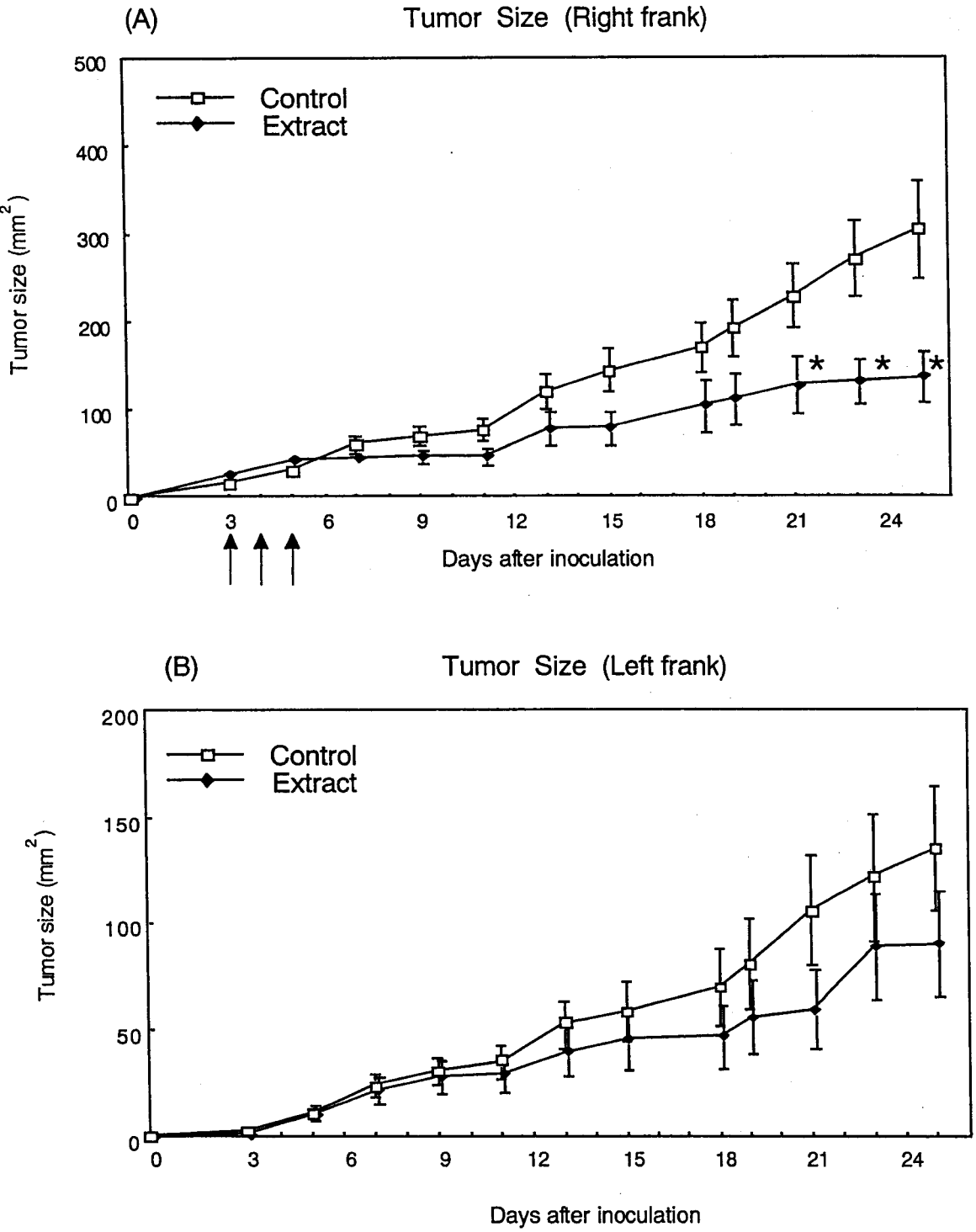

Fig. Antitumor effect of EFH-201 extract on enlagement of Meth-A fibrosarcoma transplanted in mice(n=10). Meth A cells were inoculated with 1×10^6 cells into the right(A) and 2×10^5 cells into the left (B) flanks of mice (double grafted tumor system). Then, 1 mg of the extract was injected intratumorally in the right flanks on days 3, 4 and 5 (at arrows). The tumor sizes were measured at indicated intervals.

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t-test * $P<0.05$ or ** $P<0.01$. (n=) : number of mice detected for SM-resistent *E. coli* in the feces.

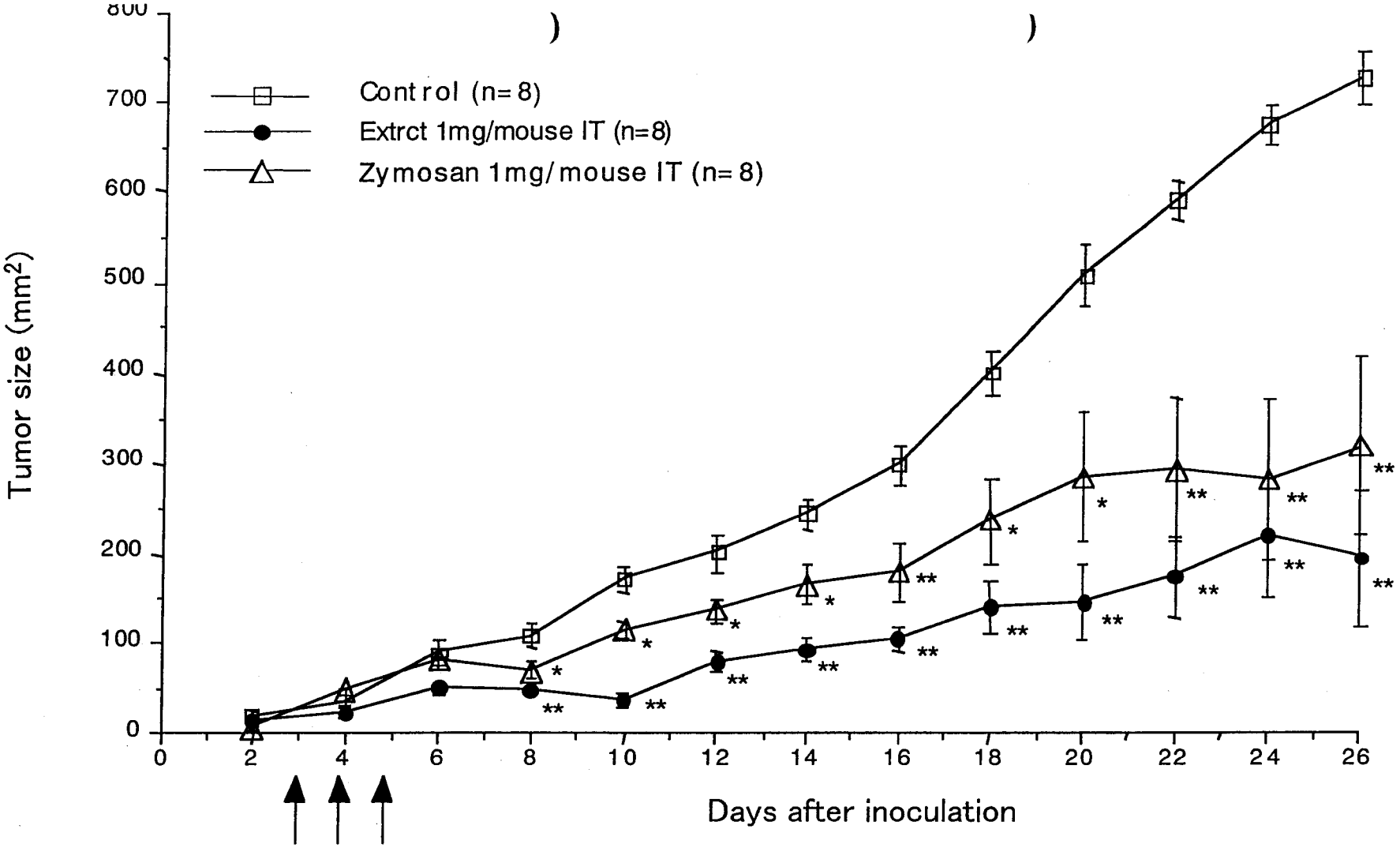

Fig. Antitumor effect of EFH-201 extract on enlargement of Sarcoma-180 in mice. Sarcoma-180 (1.7×10^6 cells) was inoculated subcutaneusly into the abdormens of mice (n=8) on day 0, and then 1 mg of the extract or zymozan injected intratumorally days 3, 4 and 5 after tumor inoculation (shown by arrows in this figure). The tumor size was measured at indicated intervals.

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Aspirin aluminium	500	15.9±4.6*	51.9

Mice were injected with diluted acetic acid one hour after oral administration of the EFH-201 extract or of aspirin aluminium. Then minutes later, the number of writhing mice were counted for 10 minutes in each mouse.
* $P<0.05$ and ** $P<0.01$ (: Significantly different from control.)

Table Effect of FH-201 on the number of *E. coli* NB-101 in livers of immunocompomised mice with the intravenous challenge

Group of mice	CY treatment	Number of *E. coli* in livers (log/g)		
		Time(hour) after administration of *E. coli*		
		24 hr	48 hr	72 hr
Control	+	6.3±0.7	6.9±0.6	6.2±0.5
EFH-201	+	4.8±0.8	4.5±0.5 *	4.4±0.6 *
Conventional	−	4.2±0.4 *	2.7±0.3 **	— #

Mice were fed 10% diet for 14 days, and then injected intraperitoneally with CY 5 mg. The compromised mice were infected intravenously with 1.0×10^7 cfu of *E. coli* NB-101. Each value was as mean± SE of 5 mice.

* $P<0.05$ and ** $P<0.01$ (: Significantly different from control.). # Not detected.

Table Resistance of *E. coli* NB-101 and its mutant to streptomycin

Strain	SM concentration (μg/ml)									
	800	400	200	100	50	25	12	6	3	1.5
E. coli NB-101	−	−	−	−	−	−	−	−	±	+
NB-101^{SMR}	+	+	+	+	+	+	+	+	+	+

+: god growth, ±: slight growth, −: no growth.

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Table　Antitumor effect of water soluble EFH-201 extract on enlargement of Sarcoma-180 solid tumor in mice

Group	Dose (mg/head)	Tumor weight (mean(g)±SE)	Inhibition ratio(%)
Control	Saline (×3days)	7.48±0.53	—
EFH-201 extract	1 (×3days)	2.20±0.85**	70.6
Zymosan	1 (×3days)	3.58±1.16**	52.2

Tumor cells (1.7×10^6 cells/mouse) were inoculated subcutaneously to the flanks of each mice on day 0. Two samples were injected intratumoraly on day 3, 4 and 5 vspectively. The solid tumors were resected from mice on day 26.
** $P<0.01$ (: Significantly different from control.)

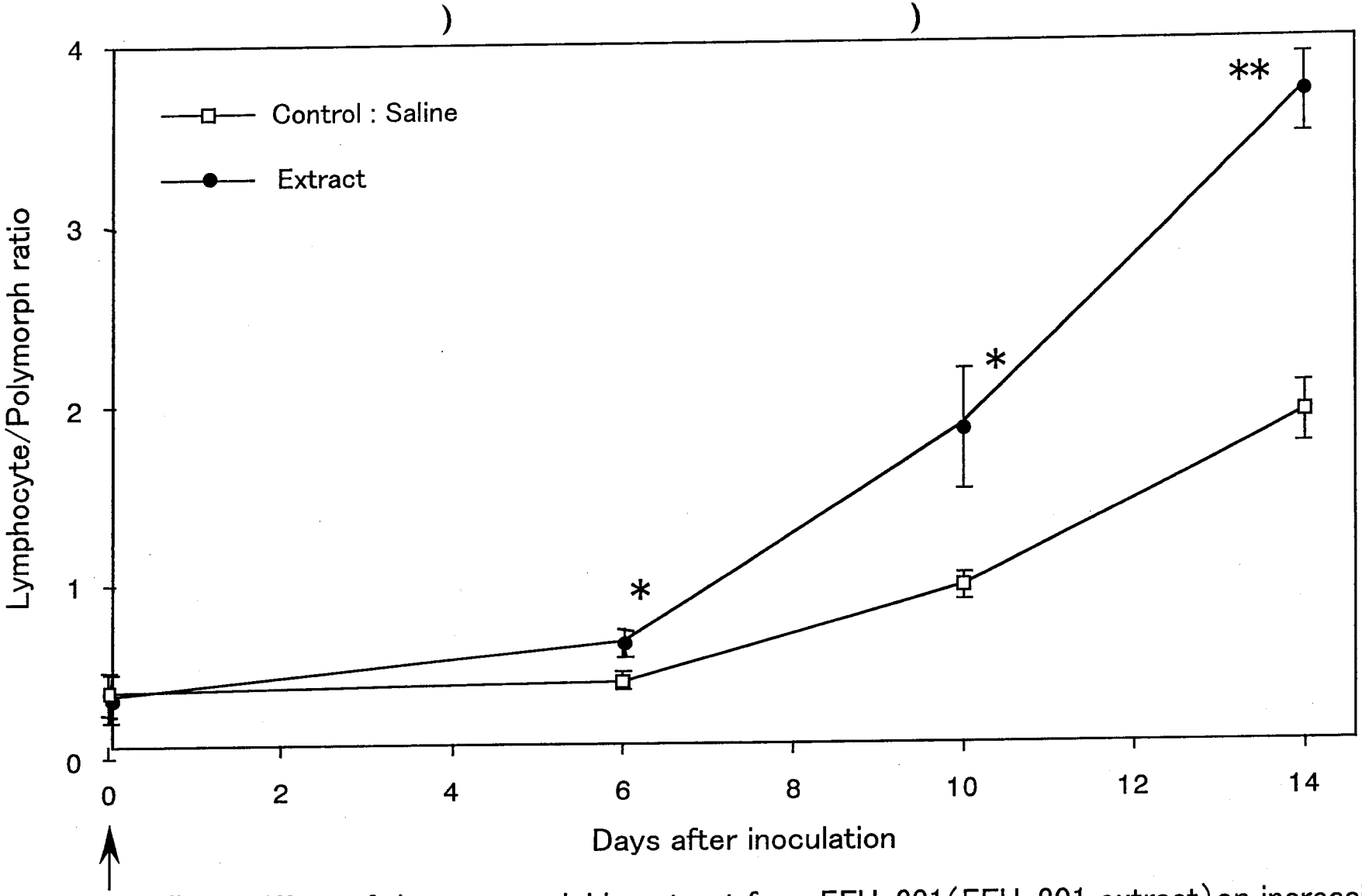

Fig. Effect of the water soluble extract from EFH-201 (EFH-201 extract) on increasing the ratio of blood lymphocyte/polymorph in mice.
Neonatal mice were injected intraperitoneally with 200 μ g of EFH-201 extract immediately after birth. L/P ratio was calculated before and after its administration (6,10 and 14 days).
* $P<0.05$ and ** $P< 0.01$ (: Significantly different from control)

Table Antitumor effect of water soluble EFH-201 extract onenlargement of Meth A solid tumor in BALB/c mice

Sample (n=10)	Dose (mg/head)	Tumor weight (mean(g)±SE)	Inhibition ratio(%)	Complete regression
Control	Saline (×3days)	7.48±0.53	—	0/10
EFH-201 extract	1 (×3days)	0.64±0.21*	56.4	1/10

Tumor cells(Meth A fibrosarcoma) were inoculated subcutaneously to the abdomen of each mouse on day 0. Two samples were injected intratumorally into the right flank on days 3, 4 and 5 vspectively. The solid tumors were resected from mice on day 26.
* $P<0.05$ (: Significantly different from control.)