Inhibitory Effects of Symbiotic Lactobacterium- and Yeast-Fermented Soy Extract on Tumor Metastasis and Allergic Reaction

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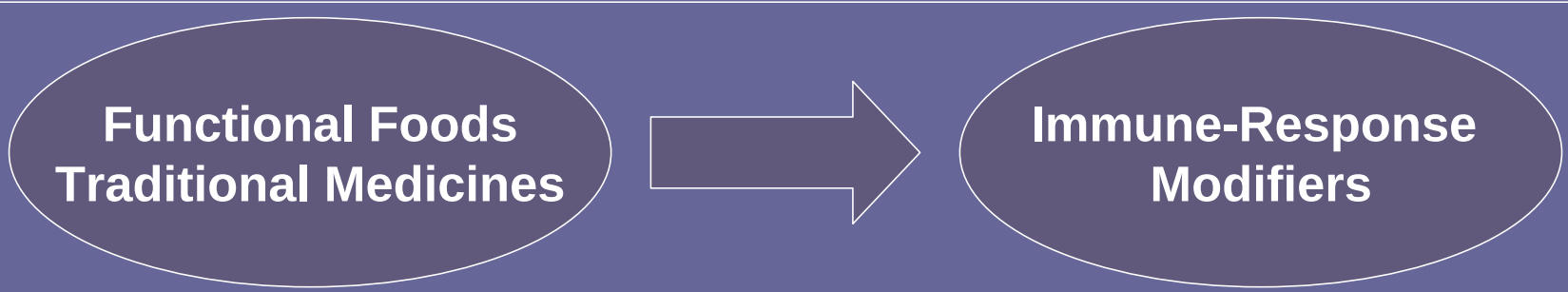
Introduction

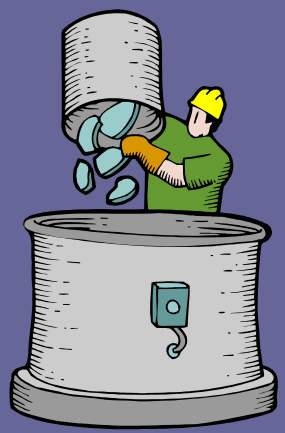
- Recently, all over the world face to serious health problem. Patients of neoplasm, allergy, and virus infection involved in immune systems are increasing continuously, since our immune systems are getting worse with daily life, food and endocrine disrupting chemicals. In the course of our studies for developing immune-response modifiers from functional foods including fermented materials and traditional medicine, we found that symbiotic Lacto bacterium- and Yeast-fermented soy extract (LYS) inhibited tumor metastasis and allergic reaction by the modulation of immune systems.



Symbiotic Lacto bacterium- and Yeast-fermented Soy Extract (LYS)

Soy (Not genetically modified)

1) Water, *r.t.*, 24 h
2) Homogenize
3) 100℃, 1h
4) Filtration



Soy Extract

Symbiotic Fermentation

1) Enzyme Reaction (Cellulase, Amylase, Protease)

2) Fermentation
Lacto Bacterium; E. faecalis, L. helveticus, L. casei, L. sp
Yeast; Saccharomyces cereviciae

1) 30℃, 4 d
2) 100℃, 1h
3) Freeze dry

Symbiotic Lacto bacterium- and Yeast-fermented Soy Extract (LYS)

Apply for various Assays

View
Activities of LYS

- Gastro protection (Inhibition of gastric lesions, ~1g/kg rat or mouse)
- Antiflatuents (Regulation of intestinal function)
- Anti-diabetes (①Suppression of high blood glucose level,② Inhibition of aldose redactase: improvements of complication)
- Control of immune systems (anti-allergy, immune-response activator)

Effect of LYS on Tumor Metastasis and Proliferation ①

Female C57/BL6 (4 weeks)

1 week

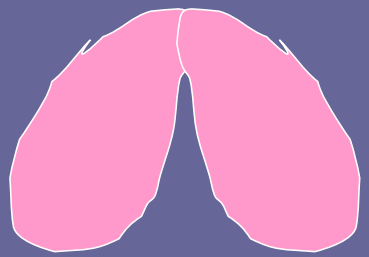
Normal diet
+
Water
or
1%, 2% LYS water
or
1%, 2% lipoprotein water

Injection (*i.v.*)
B16F10 melanoma, 10^5cells/mouse

2 weeks

Same condition as above

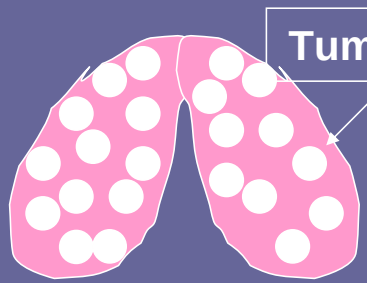
Count tumor colonies in lung



Lung

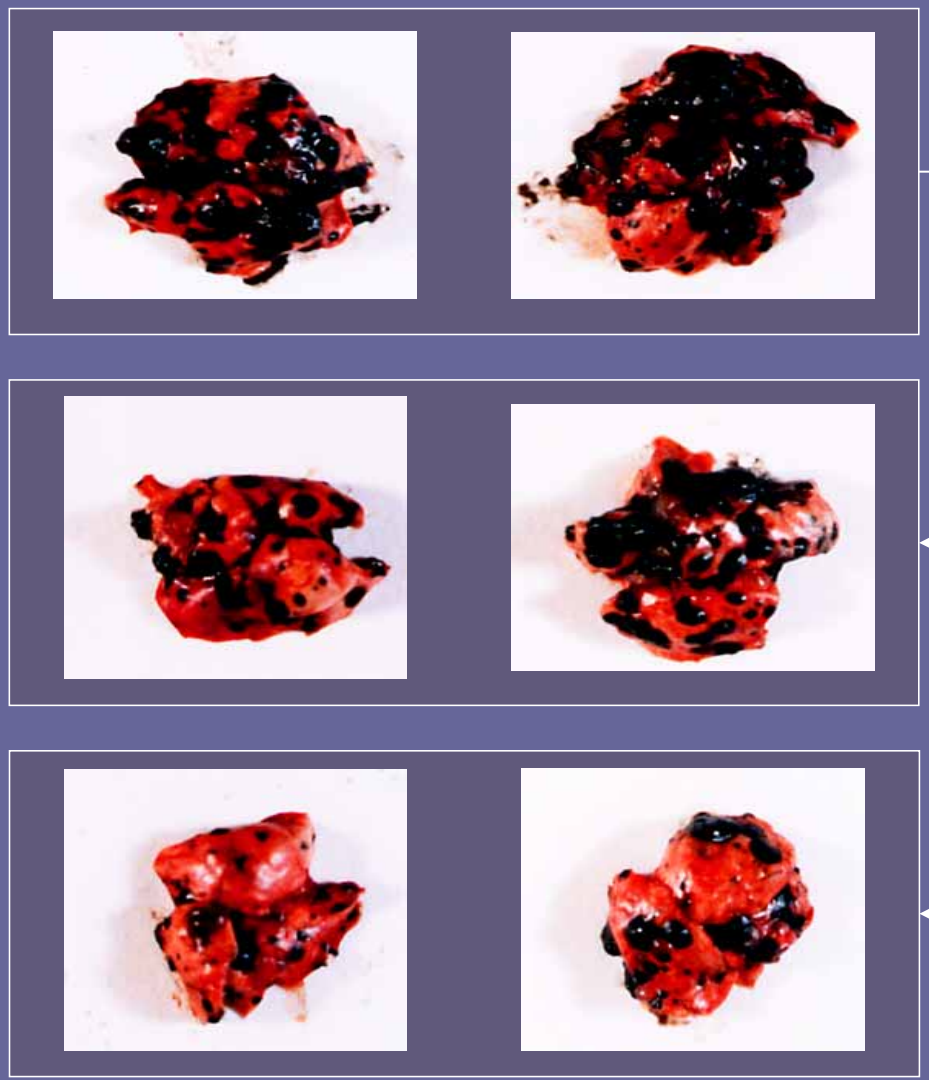
Melanoma

Injection



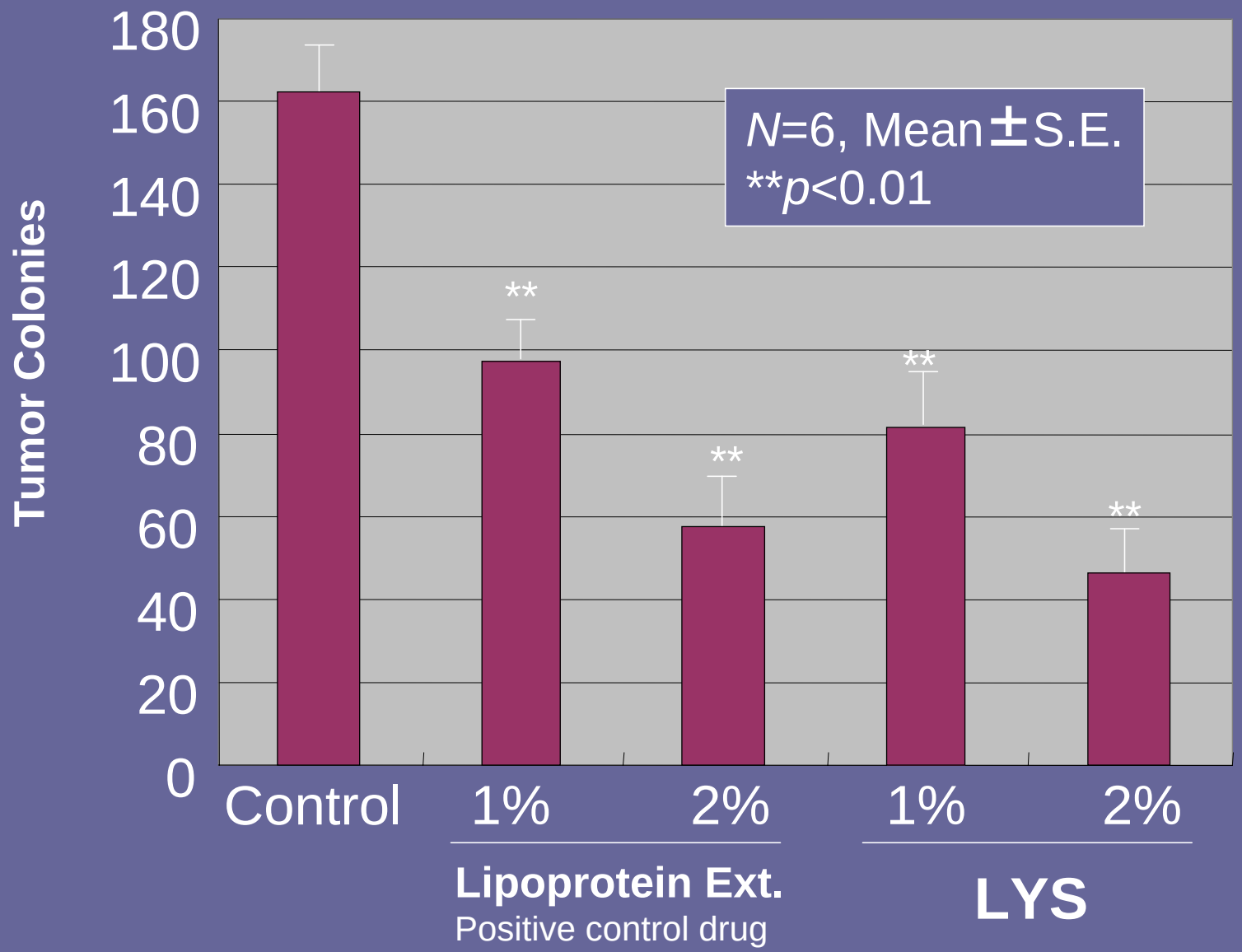
Metastasis and Proliferation

Effect of LYS on Tumor Metastasis and Proliferation ②



**$p<0.01$

Effect of LYS on Tumor Metastasis and Proliferation ③



Effect of LYS on Macrophage Activation ①

Male ddY mouse (30 g)

Wash peritoneal with PBS

Selection (Preculture)

Mouse peritoneal macrophage

LYS (~300 μg/ml)
or
LPS (10 μg/ml, positive control)

37℃, 20 h

Measurement of Nitric oxide (NO) in the culture medium by Griess reagent

①Collected from mouse peritoneal macrophage

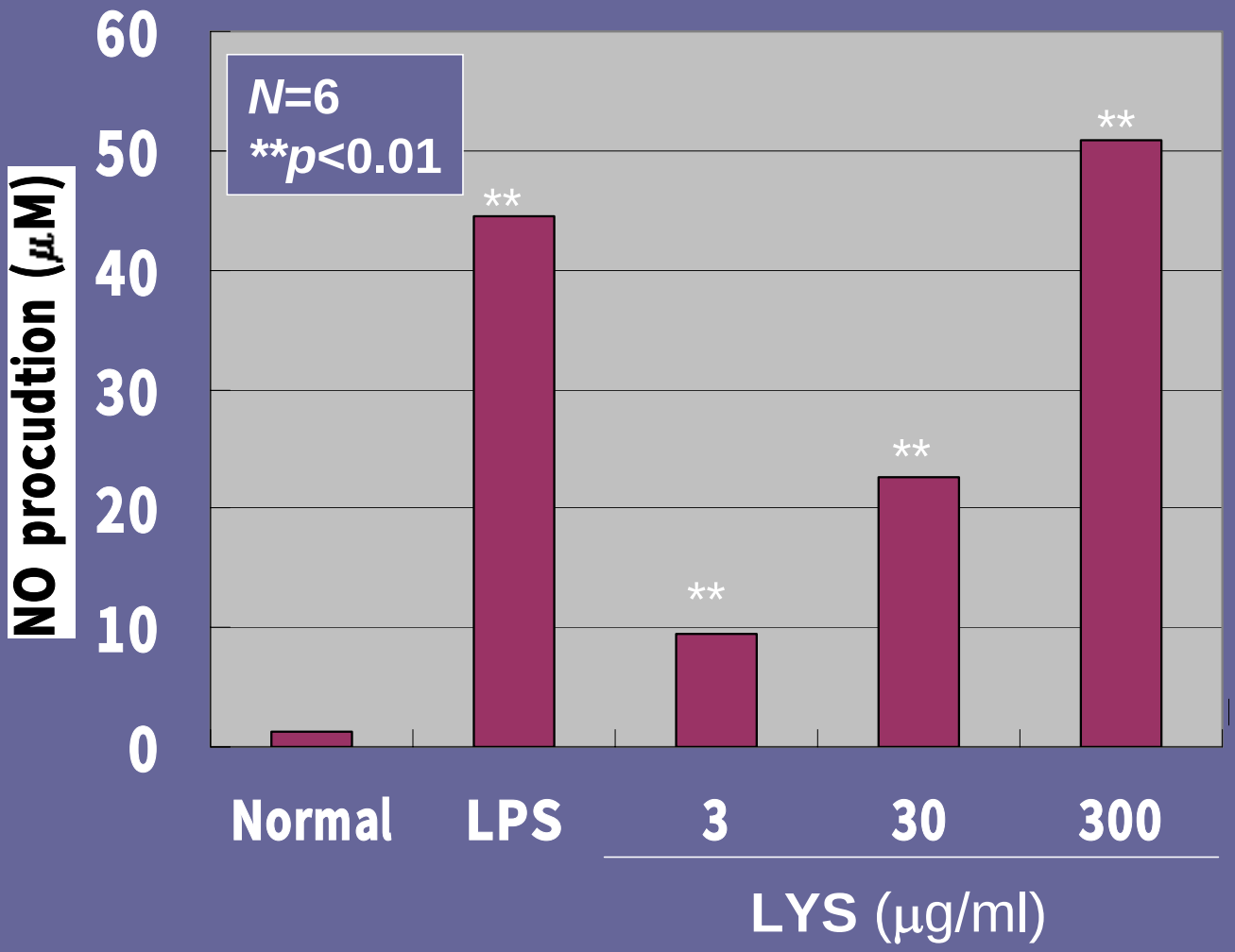
②Primary culture LYS or LPS

Measurement of NO production by Griess reagent

Activation Marker

LPS; lipopolysaccharide from *S. aureus*

Effect of LYS on Macrophage Activation ②



Effect of LYS on Macrophage Activation ③

Mouse peritoneal macrophage

LYS (~300 μg/ml)
or
LPS (10 μg/ml)
37℃, 1 h

Fluorescent Beads
for phagocytic activity

37℃, 1 h

Trypan Blue (quenching)

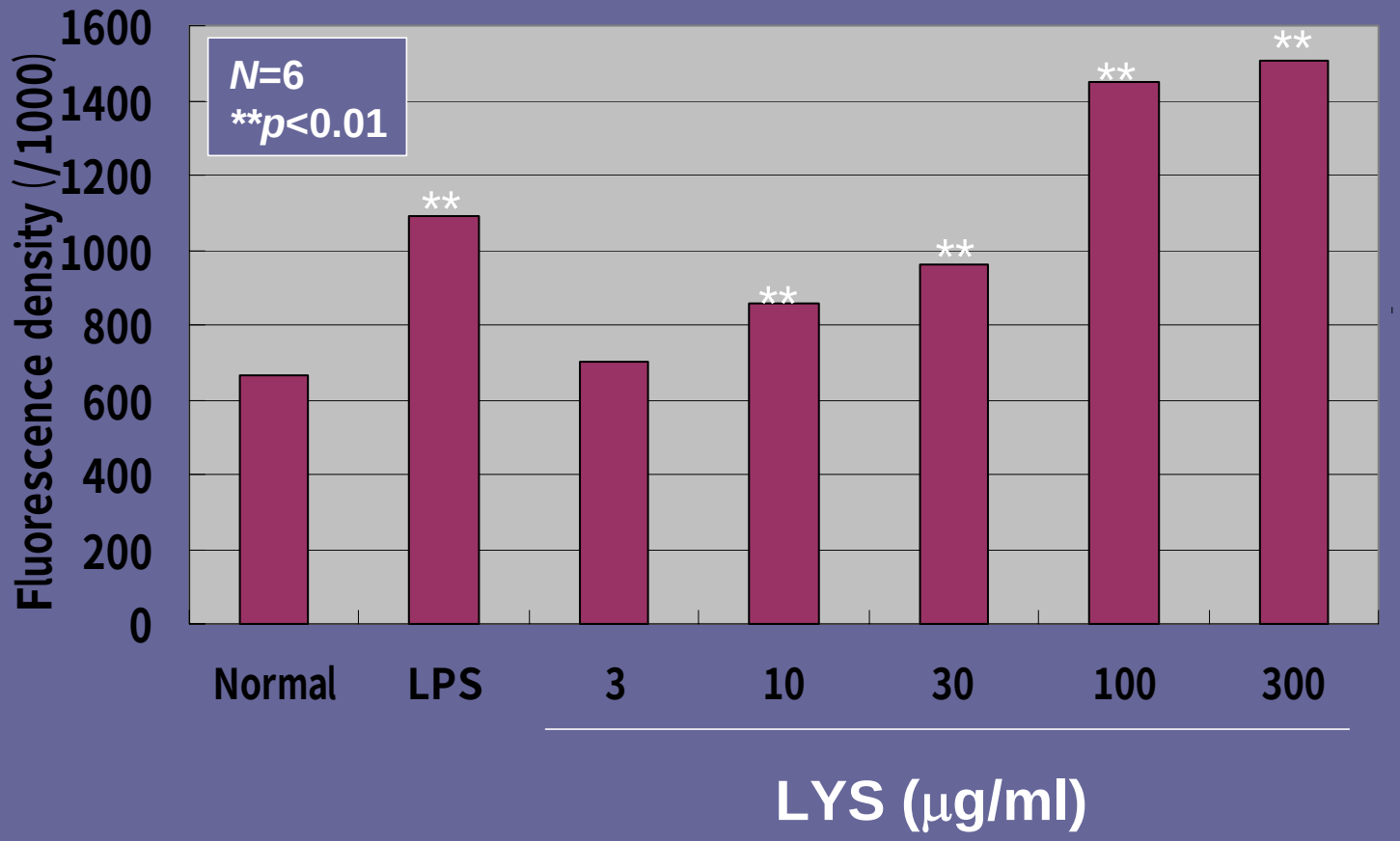
Measurement of fluorescence density
of activated macrophage (Ex.532 nm, Em.526 nm)

①Collected from mouse peritoneal macrophage

②Primary culture LYS or LPS
③Bio-Beads (Fluorescence)

Measurement of fluorescence density

Effect of LYS on Macrophage Activation ④



Effect of LYS on Allergic Reaction ①

Male SD rat（120 g）

↓

Anti-DNP IgE

Injection

↓

LYS, 1000mg/kg/day, 3times, *p.o.*

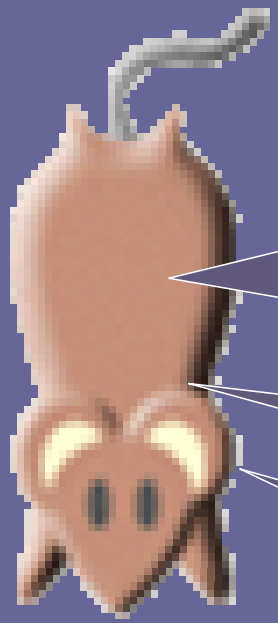
↓

1% Evans blue-saline containing 0.75 mg DNP-BSA, *i.v.*

30 min

↓

Measurement of the pigmentation area.



Dorsal skin sites Injected intradermally with DNP-IgE

LYS, *p.o.*

DNP-BSA saline, *i.v.*

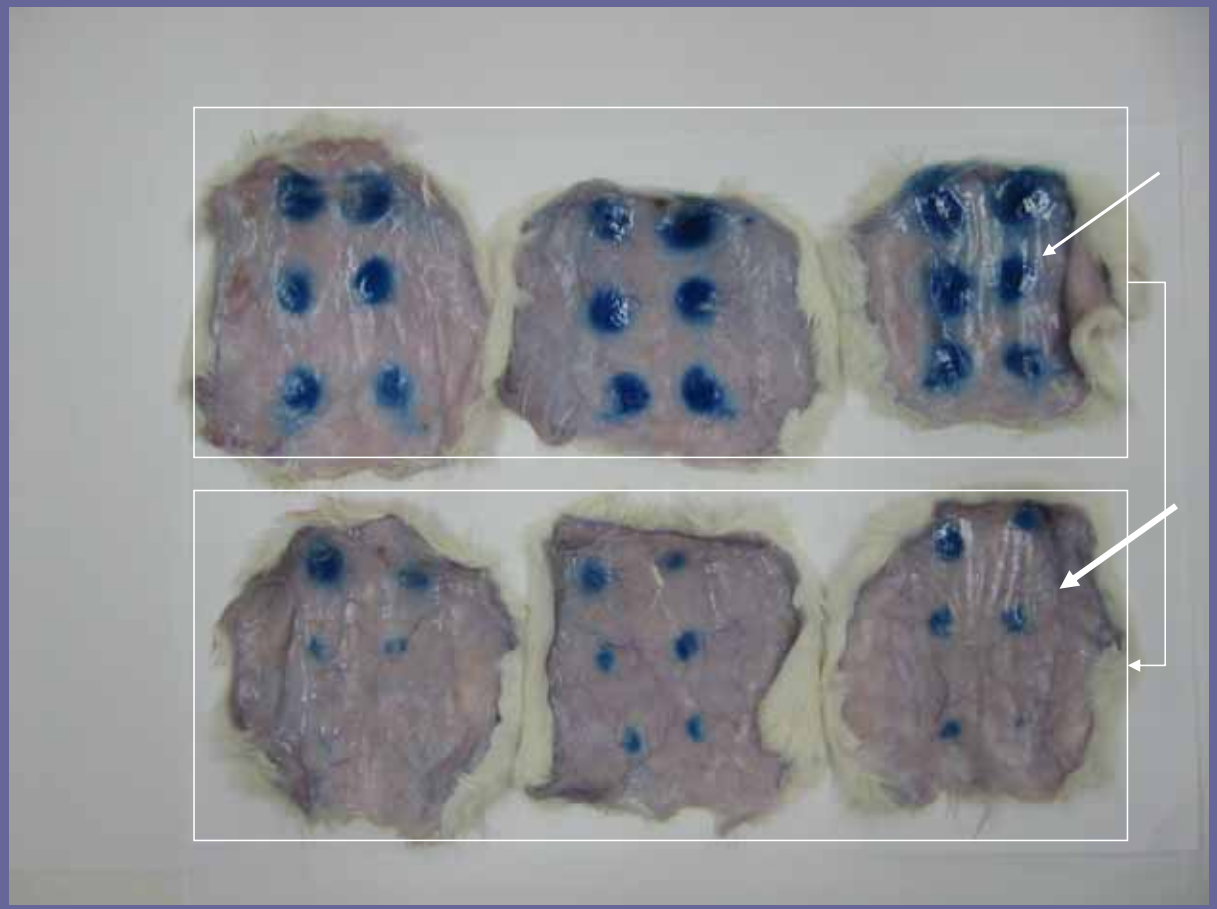
Measurement of the pigmentation area.

Effect LYS on Allergic Reaction ②

Control

LYS

1000mg/kg/3times



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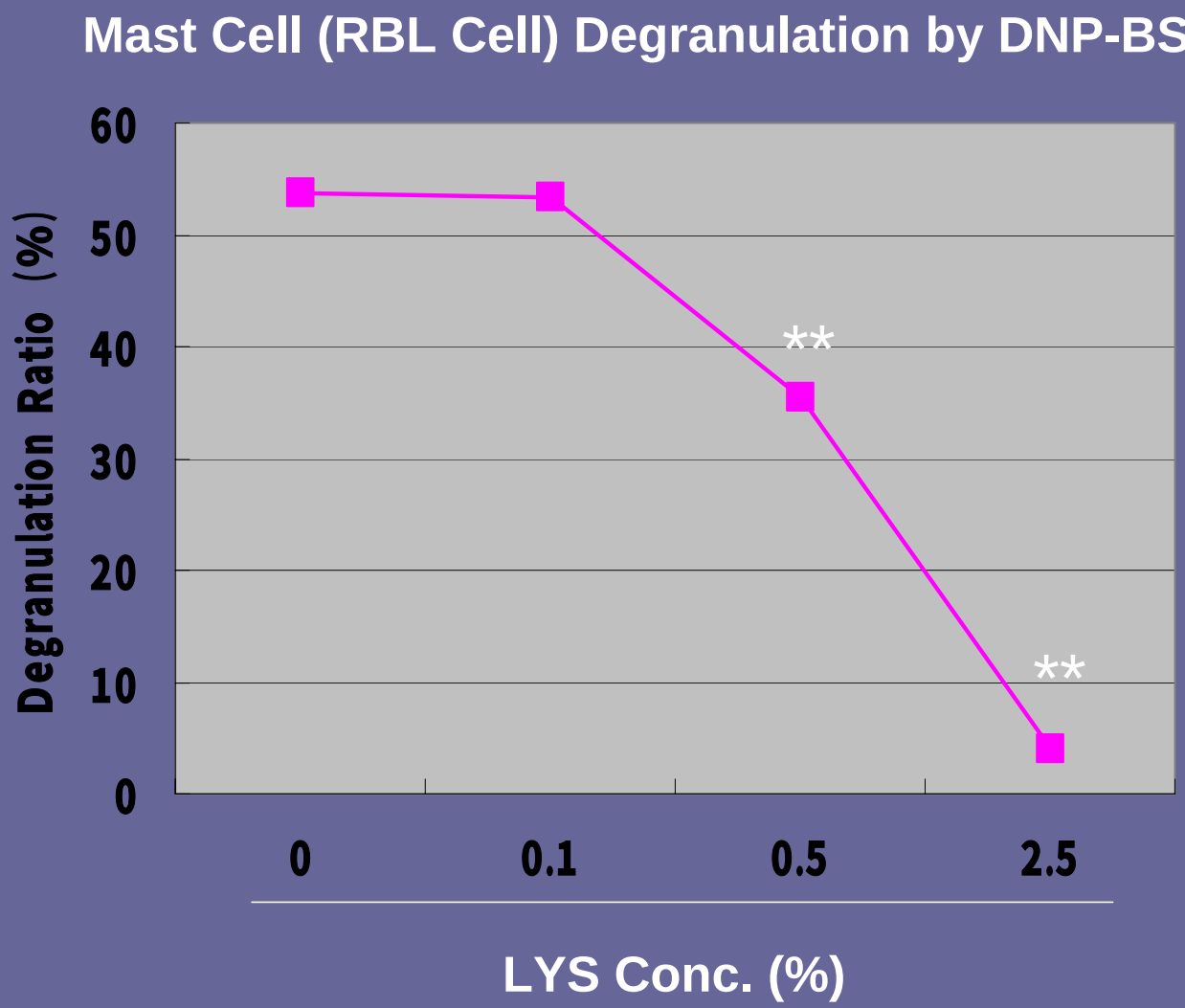
***N*=6**

****p*<0.01**

Effect of LYS on Allergic Reaction ③



Conclusion

- By in vivo administration of LYS, melanoma metastasis in lung was significantly suppressed depending on its dose dependence. At dose level of 2% LYS, colonies of melanoma were decreased 1/3-fold. And anti-metastasis activity of LYS was 20% stronger than positive control drug of lipoprotein.
- Passive cutaneous anaphylaxis was significantly suppressed by LYS for inhibiting mast cell degranulation and decreased 1/4-fold.
- These data indicates that LYS inhibits tumor metastasis and IgE-mediated type I allergy by modulating the immunocompetent cells activation.
- The LYS, symbiotic Lacto bacterium- and Yeast-fermented soy extract, is considered to be one of the immunomodulator.

Anti-Tumor Metastasis and Anti-Allergy of LYS

LYS
Symbiotic Lacto Bacterium- Yeast-fermented Soy Extraxt

Immune System
(Intestinal Immune System)

Modulation

Immunocytes (Macrophage, leukocyte….) Modulation

Activation

Inhibition

Inhibition of Tumor Metastasis and Proliferation

Inhibition of Allergic Reaction

Immunomodulator, Immune-response modifiers