

# Macrophages IL-1 M11C (non-lectin components)

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<sup>1</sup>, <sup>1</sup>, <sup>1,2</sup>, <sup>1</sup>, <sup>1</sup>, <sup>3</sup>, <sup>4</sup>, <sup>2</sup>, <sup>1†</sup>

## The effect of Korean mistletoe extract M11C (non-lectin components) on IL-1 release and expression from macrophages

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**Background:** Korean mistletoe (*Viscum album*) extract has been found to possess immunostimulatory activity. In this study, Korean mistletoe extract, M11C (non-lectin components), was used to know whether this extract might activate mouse peritoneal macrophages to produce interleukin 1 (IL-1). **Methods:** Hemagglutination assay was carried out to examine whether M11C contained a lectin or not. To know the effect of M11C on the production of IL-1, the macrophages were treated by the M11C, and then collected the supernatant (M11C stimulated macrophages-conditioned media; MMCM). MMCM was analyzed for the IL-1 quantification and mRNA expression by means of ELISA and RT-PCR, respectively. **Results:** Maximum effective dose and time of M11C on IL-1 production from macrophages were 20  $\mu\text{g}/\text{Ml}$  and 8 hours, respectively. This ELISA data was reconfirmed by immunoblotting assay, indicating that M11C is a good candidate for an immunomodulator. The dose and time dependent effects of M11C on the expression of IL-1 mRNA from macrophages was also shown in expression of mRNA detected by RT-PCR. Treatment dose and time for the maximum expression of IL-1 mRNA were 20  $\mu\text{g}/\text{Ml}$  and 4 hours, respectively. Maximum gene expression of IL-1 was much earlier than maximum production of it. **Conclusion:** As results, Korean mistletoe extract, M11C, may be used for an immunomodulator. This will be able to make up for and solve the problems caused by existent immunoagent with many adverse effects through many other studies in future including one molecule extraction.

**Key Words:** Korean mistletoe, non-lectin components, M11C, macrophages, IL-1 release, IL-1 expression

(1-4). helper T cell, NK cell  
 hematopoietic cell, fibroblast, vascular endothelial  
 cell (15, 16).  
 (1). 가  
 1990 가  
 가  
 (2-7). macro-  
 phage IL-1  
 가  
 macrophage IL-1 가  
 가  
 polysaccharides, oligo-  
 saccharides, amines, alkaloids (8-12).  
 T  
 macrophages macro-  
 phages (12).  
 가  
 macrophage  
 , endotoxin, mitogen, virus  
 (12).  
 cytokine macrophage가  
 (13, 14).  
 Macrophage가 cytokine  
 interleukin 1(IL-1), interleukin 6(IL-6), interleukin 12  
 (IL-12), tumor necrosis factor (TNF- ), interferon-  
 (IFN- ) (13, 14).  
 macrophage TNF- IL-1  
 가 cytokine  
 IL-1 , macrophage  
 macrophage B lymphocyte,  
 dendritic cell, fibroblast, keratinocyte, Langerhans cell,  
 neutrophil, astrocyte, epithelial cell, endothelial cell  
 (15, (12). M 11C  
 16). IL-1 (17-19). IL-1 ( 82A, )  
 1. ,  
 RPMI-1640 MEM,  
 fetal bovine serum (FBS), L-glutamine acid, penicillin-  
 streptomycin, trypsin-EDTA Gibco-BRL (Grand Island,  
 NY, USA) , culture plates,  
 flasks, tube Falcon(Franklin Lakes, NJ,  
 USA) IL-1 enzyme-  
 linked immunosorbent assays (ELISA) kit immuno-  
 blotting Chemicon (Temecula, CA,  
 USA) (RT-  
 PCR) Takara (Otsu, Shiga, Japan)  
 . Chloroform, hexane, methanol, ethanol,  
 isopropanol Merck (Berlin,  
 Germany)  
 0.1%  
 mouse  
 ( , )  
 22±  
 1 , 55±5%, 150 Lux Macro-  
 phages Balb/c  
 2. M 11C  
 M 11C  
 (12). , M 11C  
 2

4 ( , ) .  
 30 ,  
 chloroform hexane  
 (60 $\mu$ m 0.45 $\mu$ m; Millipore, Bedford, MA 01730, USA) ,  
 polymyxin-B column (Biorad, Hercules, CA, USA)  
 lipopolysaccharide (LPS) PBS  
 ( M11C ). M11C LPS  
 Limulus ES II kit (Wako, Osaka, Japan)  
 , 42 endotoxin units (EU)/ml (data  
 not shown). 가 LPS  
 350 EU/ml (20), M11C 1/8  
 가 가 .  
 3. (hemagglutination test)

M11C  
 (12, 21, 22).  
 B  
 PBS  
 3 2%가 PBS .  
 solution M11C  
 negative control PBS, positive control  
 Korean mistletoe lectin KML 가 96 well  
 microtiter plate 1 M11C  
 .

4. Mouse macrophages  
 Macrophage  
 (12, 23). , RPMI-1640  
 24 well plate macrophages plating .  
 macrophages 1  
 macrophages가 ,  
 Wright and  
 Giemsa stain macrophages  
 98% (data not shown). M11C  
 macrophage plate incomplete RPMI-1640 medium  
 , serum free RPMI-1640 medium  
 (+penicillin-streptomycin, +L-glutamine) 1 M  
 . Macrophages M11C 가 , 37  
 medium -20

## 5. Sandwich ELISA

IL-1 ELISA kit (Chemicon, Temecula, CA, USA)  
 . IL-1 standard samples 96 well (precoated  
 with rat anti-mouse IL-1 ) 100  $\mu$ l .  
 primary antibody rabbit anti-mouse TNF- 25  
 $\mu$ l 가 , 4  
 , wash buffer . 100  $\mu$ l second  
 antibody goat anti-rabbit conjugated alkaline phosphatase  
 well 가 45 .  
 wash buffer , vacuum  
 . color reagent  
 well , well  
 stop solution 50  $\mu$ l  
 . 490 nm ELISA reader (Bio-Tek, Highland park,  
 Vermont, USA) IL-1  
 (24).

## 6. Western blotting

Western blotting  
 (12, 25). , MMCM sample  
 buffer 16% separating gel 0.1%  
 SDS-PAGE .  
 gel nitrocellulose membrane (NC; Schleicher and Schuell, Verkaufsleiter, Germany)  
 , NC blocking buffer (5% nonfat dry  
 milk in TBS-T buffer)  
 . TBS-T buffer , rabbit  
 anti-murine IL-1 polyclonal primary antibody 가  
 . hybridization incubator (Robbins,  
 CA, USA; speed 10rpm) 4 12  
 . TBS-T buffer , 2 (goat  
 anti-rabbit IgG horseradish peroxidase conjugated affinity  
 purified antibody) 가 hybridization incubator  
 4 3 , TBS-T  
 . NC ECL(peroxidase substrate, Amersham,  
 Cleveland, Ohio, USA) 5 .  
 , 10W safety lamp (Kodak, Rochester, NY,  
 USA)가 x-ray film(Agfa, Devaert,  
 Belgium)) x-ray film developing,

Table 1. Primers used in RT-PCR

Target mRNA	Primer sequences	Product size (bp)
IL-1	sense: 5'-ATGGCAACTGTTCTGAACTCAAC-3' anti-sense: 5'-CAGGACAGGTATAGATTCTTTCTTT-3'	563
-actin	sense: 5'-GGAGAAGATCTGGCACCAACC-3' anti-sense: 5'-CCTGCTTGCTGATCCACATCTGCTGG-3'	840

washing, fixing, washing IL-1  
band .

7. (reverse transcription -  
polymerase chain reaction)  
(RT-PCR)  
(12), kit  
kit(Takara, Otsu, Shiga, Japan)  
, Trisol(Gibco-BRL, Grand  
Island, NY, USA) total RNA .  
2 µg total RNA (total volume: 50 µl;  
10 µl of 5x RAV-RTase buffer, 20 µl of 2.5 mM dNTP,  
0.02 µM of IL-1 or -actin anti-sense primer, 10  
units of RAV-RTase) 42 1  
IL-1 -actin cDNA .  
cDNA (total volume: 50  
µl; 5 µl of 10x Taq buffer, 4 µl of 2.5 mM dNTP, 1  
µl of 10 pmoles/µl IL-1 or -actin sense or anti-sense  
primer, 8 units of Taq) IL-1 -actin  
RT-PCR . 1% agarose  
gel . primer  
Table 1 .

8.  
mean ± SD ,  
group PC-SAS Excel  
T- . p 0.05  
가 .  
1. M11C  
(non - lectin )  
M11C가 agglutinin 가

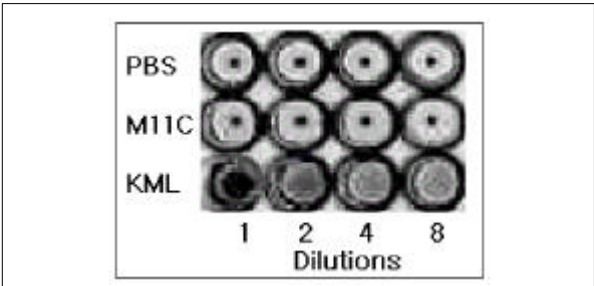
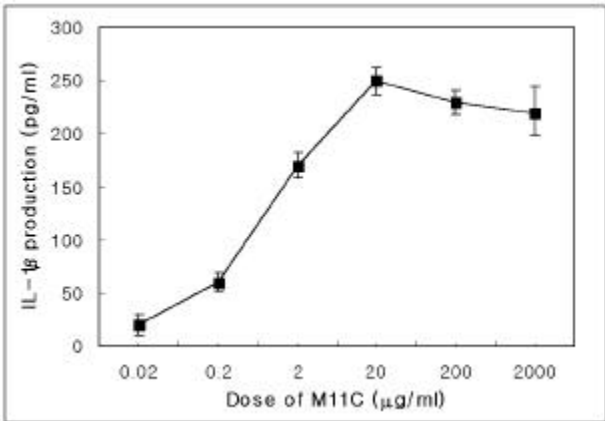
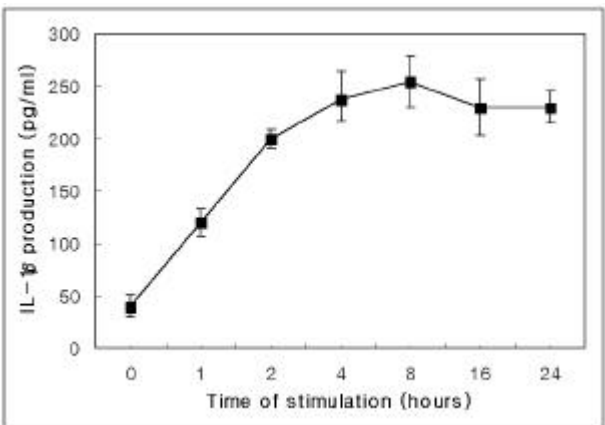


Fig. 1. The effect of Korean mistletoe crude extract (M11C) on agglutination of RBC. 1, 2, 4 and 8 express 1, 2, 4, 8 multiple dilutions, respectively. PBS, M11C, and KML represent negative control, Korean mistletoe crude extract, and Korean mistletoe lectin, respectively. The result is one of the four experiments. All other details are as described under "Materials and Methods".

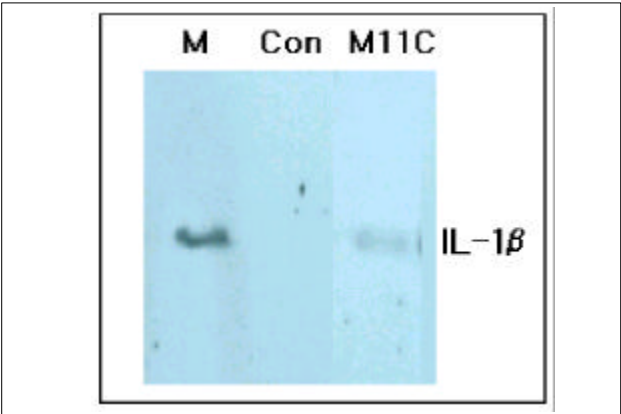
(Fig. 1) . M11C  
negative control PBS  
. Positive control  
KML ,  
8 µg/ml  
(data not shown). ,  
M11C agglutinin  
.  
2. M11C macrophages IL - 1  
M11C macrophage 8  
M11C 0.02 µg/ml medium  
alone IL - 1 (data not  
shown). ED50 2 µg/ml . 가  
가 IL - 1 가 M11C  
20 µg/ml 245 ± 6 pg/ml IL - 1  
IL - 1 0.02  
µg/ml



**Fig. 2.** The dose dependent effect of Korean mistletoe crude extract (M11C) on interleukin-1 (IL-1) release from macrophage. Bars represent the mean  $\pm$  S.E.M for three experiments with triplicate. All other details are as described under "Materials and Methods".



**Fig. 3.** The time dependent effect of Korean mistletoe crude extract (M11C) on interleukin-1 (IL-1) release from macrophage. Bars represent the mean  $\pm$  S.E.M for three experiments with triplicate. All other details are as described under "Materials and Methods".



**Fig. 4.** Immunoblot analysis of conditioned media obtained from Korean mistletoe crude extract (M11C)-treated macrophage using polyclonal antibody of rabbit anti-mouse IL-1. M and Con represent marker and control (medium alone) for IL-1, respectively. The result is one of the five experiments. All other details are as described under "Materials and Methods".

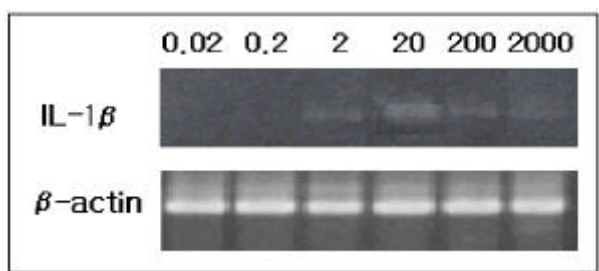
M 11C 20 µg/ml  
macrophage (Fig. 2-3).  
IL-1 가 45 ± 5 pg/ml  
8 250 ± 20 pg/ml 5  
가 ,  
(p<0.001). 8 plateau  
(Fig. 3).  
M 11C macrophage  
20 µg/ml 8 (Fig. 2-3). IL-1  
M 11C  
TNF- 1/10 (data  
not shown; 12). ELISA (Fig.  
2-3)  
immunoblotting .  
M 11C  
macrophages ELISA  
IL-1 가  
(Fig. 4).

4. M 11C macrophages IL-1 mRNA

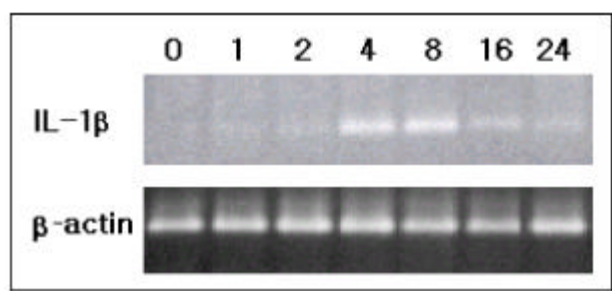
PCR

, IL-1 mRNA

(p<0.001). plateau  
(Fig. 2). IL-1  
M 11C 20 µg/ml  
macrophage TNF-  
M 11C (data not shown).  
3. M 11C macrophages IL-1



**Fig. 5.** The dose dependent effect of Korean mistletoe crude extract (M11C) on interleukin-1 (IL-1) mRNA expression from macrophage. Expression of mRNA was detected by RT-PCR. Equivalent quantities of mRNA were used since similar amounts of  $\beta$ -actin were expressed in each specimen. 0.02, 0.2, 2, 20, 200, and 2000 represent 0.02  $\mu\text{g/ml}$ , 0.2  $\mu\text{g/ml}$ , 2  $\mu\text{g/ml}$ , 20  $\mu\text{g/ml}$ , 200  $\mu\text{g/ml}$ , and 2000  $\mu\text{g/ml}$  of M11C dose, respectively, that were used for the stimulation of macrophage for 8 hours. The result is one of the five experiments. All other details are as described under "Materials and Methods".



**Fig. 6.** The time dependent effect of Korean mistletoe crude extract (M11C) on interleukin-1 (IL-1) mRNA expression from macrophage. Expression of mRNA was detected by RT-PCR. Equivalent quantities of mRNA were used since similar amounts of  $\beta$ -actin were expressed in each specimen. 0, 1, 2, 4, 8, 16, and 24 represent 0 hour, 1 hour, 2 hours, 4 hours, 8 hours, 16 hours, and 24 hours of culture time, respectively, that were used for the stimulation of macrophage with M11C (20  $\mu\text{g/ml}$ ). The result is one of the five experiments. All other details are as described under "Materials and Methods".

M11C  
M11C macrophage M11C 0.02  $\mu\text{g/ml}$   
0.2  $\mu\text{g/ml}$  IL-1 mRNA  
(Fig. 5). ELISA  
M11C 0.2  $\mu\text{g/ml}$  가 macrophage  
IL-1 (Fig. 2, 4).

sensitivity , PCR  
ELISA sensitivity 가  
M11C 2  
 $\mu\text{g/ml}$  , 20  $\mu\text{g/ml}$  . M11C  
IL-1  
M11C 20  $\mu\text{g/ml}$  ,  
IL-1 IL-1 mRNA  
가 (Fig. 2, 5).

### 5. M11C macrophages IL-1 mRNA

IL-1 mRNA  
 , M11C 20  $\mu\text{g/ml}$   
macrophage , 1 2  
가 4 8  
(Fig. 6).  
가 . M11C macrophage  
20  $\mu\text{g/ml}$   
4 8 (Fig. 5-6). 8  
IL-1 plateau  
 , IL-1 mRNA  
(Fig. 3, 6).

가  
(1-7, 12). 가  
 ,  
가 macrophage IL-1  
KML  
M11C 가  
가 (Fig. 1).  
M11C (non-lectin com-  
ponents) .  
가

(5-6, 8-12).  
M11C  
(non-lectin components)  
(Fig. 1).  
IL-1  
RT-PCR  
, IL-1 mRNA IL-1  
M11C  
IL-1 mRNA IL-1 2  
peak  
, (Fig. 2, 3, 5, 6).  
M11C가 macrophage TNF-가  
(12). Macrophage가 TNF- IL-1  
IL-1, IL-6, IL-12가 가  
(15-19). M11C가 IL-1  
macrophage IL-1 cytokine, T- B-가  
M11C macrophage  
IL-1 가 M11C 가  
ELISA, Immunoblotting, RT-PCR M11C가 endotoxin lipopolysaccharide (LPS)  
ELISA M11C 20  $\mu\text{g}/\text{M}\ell$  8 IL-1 TNF-  
macrophages IL-1 가 가 septic shock  
(Fig. 2, 3). M11C M11C가 가  
macrophage TNF- $\alpha$  M11C  
(12). 20  $\mu\text{g}/\text{ml}$  LPS 1  $\mu\text{g}/\text{ml}$  20  
IL-1 ELISA 1/4 IL-1 (data not  
immunoblotting shown). M11C 20  $\mu\text{g}/\text{ml}$   
M11C ELISA macrophage plateau  
20  $\mu\text{g}/\text{M}\ell$  LPS 1  $\mu\text{g}/\text{ml}$   
8 (Fig. 4). , ELISA plateau가 (data not  
M11C가 macrophage IL-1 shown). M11C가 IL-1  
. ELISA IL-1  
M11C macrophage IL-1  
TNF- $\alpha$  1/10 (data not M11C (inflammatory media-  
shown). ML I, ML II, ML III tor)  
IL-1 2 3 (non-lectin)  
(26). M11C가  
macrophage TNF- $\alpha$ , IL-1 $\alpha$ , IL-1 macrophages TNF-  
(27). (12) IL-1 가  
IL-1 M11C가  
cytokine 가  
(28). M11C  
Macrophages가 M11C IL-1  
signal transduction

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