

Platycodin D Improves the Immunogenicity of Newcastle Disease Virus-Based Recombinant Avian Influenza Vaccine in Mice

Yong Xie), Hong-Xiang Sun*), Duo Li)

A , C A A E E , 310029, . Cu
(: 86-571-86971091; : 86-571-86971091; - : @ . .)
) B E , 310029,
) T Cu , 350108,
. Cu

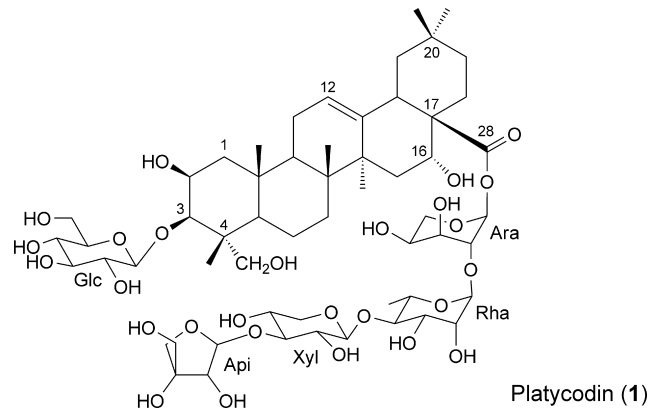
T
(A) T
(1),
Platycodon grandiflorum,
(- 5) . C 1 A (C A)-, -
(), 1, 2, 2 (P<0.05, P<0.01, P<0.001)
- 5.T A T1T2 (-γ -10)
(T- A A-3) 1, 1
- 5 (P<0.01 P<0.001). , 1
() (P<0.05),
1 - 5 1

Introduction. A
1. (A) (A)
5 7 (A)
2 3 . 2005, 50
T A E A , A , E 4 .
5 1 5 . A
(A) 6 5 1- A 7 . 2009, 424

5 1 60% (261
 8 .T
 9 .
 10 .
 11 ,
 (A) 9 .T
 A 12 , A
 13 16 , A-
 () 17 () 18 21 .
 () A
 A 22 .
 A
 23 24 .A
 A
 ; 59 27 28 , (A) 29 , 25 26 .
 30 , 5 1 .A
 59- 31 .A
 T- T1 , C 59
 T1 32 . -21 T1
 33 .T

(1)
Platycodon grandiflorum. A 1 B
 34
 35 , T
 1
 (- 5)

2 2 ,



Results. *Effects of 1 on Splenocyte Proliferation in Mice Immunized with rL-H5.*

Fig. 1. T_H 1 (A) and T_H 2 (B) cell proliferation in mice immunized with rL-H5 and treated with 1 (50, 75, 100 μg/kg) or Quillaja saponaria (25 μg/kg) (A) or 5A (50, 75, 100 μg/kg) (B). (P < 0.01, P < 0.05, P < 0.001). A (C) and A (D) represent the control groups. T_H 1 and T_H 2 cell proliferation were measured by ³H-thymidine incorporation into DNA. Data are expressed as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

Effects of 1 on Natural Killer (NK) Cell Activity in Mice Immunized with rL-H5.

Fig. 2. T_H 1 (A) and T_H 2 (B) cell activity in mice immunized with rL-H5 and treated with 1 (50, 75, 100 μg/kg) or Quillaja saponaria (25 μg/kg) (A) or 5A (50, 75, 100 μg/kg) (B). (P < 0.05, P < 0.01, P < 0.001). A (C) and A (D) represent the control groups. NK cell activity was measured by ⁵¹Cr release from target cells. Data are expressed as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

Effects of 1 on the Antigen-Specific Serum Antibody Response.

Fig. 3. T_H 1 (A) and T_H 2 (B) cell activity in mice immunized with rL-H5 and treated with 1 (50, 75, 100 μg/kg) or Quillaja saponaria (25 μg/kg) (A) or 5A (50, 75, 100 μg/kg) (B). (P < 0.05, P < 0.01, P < 0.001). A (C) and A (D) represent the control groups. Serum antibody response was measured by ELISA. Data are expressed as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

1/2 1.40 1.39, T_H2-
 1.01, T_H1/T_H2
 - 5/ A 0.95 0.98,

Effects of 1 on the mRNA Expression of Cytokines and Transcription Factors.
 1 A T_H1 (- γ) T_H2 (-10)
 T- A A-3
 - 5 T- C (Table 1),
 Fig. 4 Table 2. A (25 μ) 1 (75 μ) A
 T_H2 -10 A A-3 ($P < 0.01$
 $P < 0.001$), T_H1 - γ T_H1 -10
 T- ($P < 0.01$ $P < 0.001$) T_H1 -10
 A A-3 A
 ($P < 0.01$ $P < 0.001$). T_H1, T_H2 ($P > 0.05$)
 - γ T- A - 5
 - 5/A 1 T_H1 T_H2
 T- A A-3
 - 5.

Table 1. Sequences of the Specific Primers Used for RT-PCR

A)	5'-CCCACA T AAAT CAAC CAC-3'	570
- γ	5'-CAAT TTA AACAC A-3'	459
-10	5'-T AAC C ACACACT CACCT -3'	324
T-	5'-C ACCTTTT CC CTT CCA A -3'	436
A A-3	5'-AACCA TACCT TTCCCA C-3'	255
	5'-T TC CCACT AA A A-3'	
	5'- AA CACCA ACCC AAAC-3'	
	5'-ACCACT C T ACCACT C-3'	

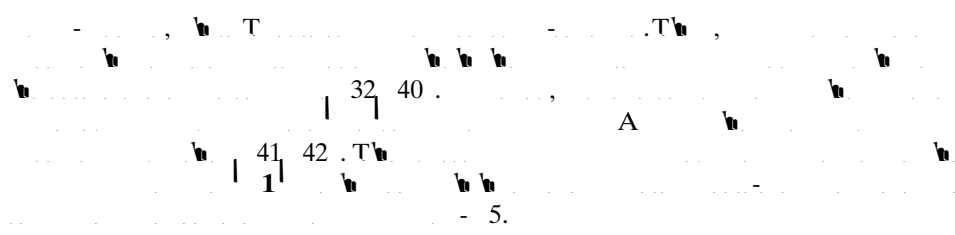
) A : -3- -

Discussion. T_H B T
 T_H
 T
 B
 36 T (A A)
 B T
 T C
 37 39 .



Fig. 4. Effects of Alum, Quil A, and **1** on the mRNA expression of GAPDH, cytokines, and transcription factors in splenocytes from mice immunized with rL-H5.

Splenocytes (5×10^6) were cultured in the presence of rL-H5 (15 μ g/ml) and Alum (200 μ g/ml), Quil A (25 μ g/ml), or **1** (75 μ g/ml) for 15 h. Total RNA was extracted and analyzed by RT-PCR for GAPDH, IL-2, IL-4, IL-5, IL-6, IL-10, T-bet, GATA-3, and T-bet. The results are shown in Table 1 (Exper. Part). T-bet and GATA-3 mRNA levels were quantified by real-time PCR. GAPDH was used as a housekeeping gene. Lane M: DNA ladder, Lane 1: Alum, Lane 2: Quil A, Lane 3: Alum + Quil A, Lane 4: Alum + **1** (75 μ g/ml).



53
54 T 55 2 56 58 . A
2
1
59 60 T
5A - 1, 2, 2 - 5. 1
- 5 1, 2, 2
1 - 5
1/ 2 1/ 2 1
- 5/1 (75 μ) 1.03 1.01, T₁T₂
- 5.
T 1 T₁ T₂
A -γ, T₁ T₁
-10, T₂
T- C . C 1 A -10,
-γ. A
T- A A-3
61 . T
T- A A-3
5, 1 A T- A A-3.
T₁ T₁ T₁ 2 -γ T-
T₂ -10 A A-3
1 - 5/1 . T
- 5 1 T₁ T₂
A
, 1 - 5
. C 1
T₁T₂ , 1

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China (. 2008BA B4B06-2), Administration of Traditional Chinese Medicine of
Zhejiang Province (. A2006 017)

Experimental Part

General. ()- (- 5),
5- (A) (A)
, 5 A (5A)
Harbin Weike Biotechnology Development Company (, C). 3-(4,5-
-2-)-2,5- -2H- (TT), A,
, RPMI 1640 , Sigma
Chemical Co. (, A). 1, 2, 2
Southern Biotech. Assoc. (B , A , A), A
Brenntag Nordic A/S (). Trizol Invitrogen (A), RevertAid
Fermentas (A), (C)
Biobasic (C), (T)₁₈ T A Sangon (C).
(C) Hangzhou Sijiqing Corp., (A)
Zhejiang Wanma Pharm Co. Ltd. (, C).
Platycodin D (Platycodigenin 3- -β-D-Glucopyranosyl-28- -[β-D-apiofuranosyl-(1→3)-β-D-xy-
lopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]; 1). (C₅₇ 92 28,
1224.5775) P. grandiflorum,
2 - 34 .T

Assay of Natural Killer (NK) Cell Activity

562
 Institute of Cell Biology, Chinese Academy of Sciences, Beijing, China
 RPMI 1640
 2 mL (Sigma), (100 μg/ml; Sigma), (100 μg/ml; Sigma), 10%
 C₂ 37° 5% C₂
 T_H 562 96- 2 × 10⁴ / RPMI
 1640 () 1 × 10⁶
 / / 50:1 T_H 20 37° 5%
 C₂ 4 50 μ TT (2 /)
 viz., TT T_H
 Eqn. 1:

$$\% = (OD_r - (OD_c - OD_e)) / OD_r \times 100 \quad (1)$$

OD_r, OD_c, OD_e

Measurement of H5Ag-Specific Antibody

5A - 1, 2, 2
 A.T_H 96- 100 μ 5A
 (1 A / 50 M 9.6) 24 4° T_H
 B 0.05% (v/v) Tween 20 (B / Tween) 5% C / B 37°
 2 A B, 100 μ 0.5% C / B
 T_H 2 37°, (3 ×
 B). A 1, 2, 2

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