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ABSTRACT

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1. M d c M

Heall B (HBV) ac i. T e a e bee i fec ed i HBV, a d 360 i i b)) e aec/ įc i fec i dide [1]. O ce i fec ed, 5. 10% ad a d 25, 90% cide bec ecrici carie [2]. Ab 25% fHBV carie die f cricacie eall, cir 1, ria ie ca ce [3]. Cricifeci 1. HBV a bec ea ar 25% f HBV ea y be y dide [4]. Ny ea e i cy e a a abefy ac e He, a i i B, a di i al i acciei e effecie ea free i HBV i fecia di ce e ce [1]. He a]] B j face a] e (HB A) ad j bed A] e c ed acciea ai HBV, a da bee ed f /d ce / ec / a a / HBV / fec / .T a eạ rec) édia a effec) e acc) e, ear 10% facc) ee). HBA faj de e rec) e e e fa } HBA a jbdje

[5]. T, a f ace i f e accial efficac a eacle

I e acce ed a f a b d f e e a e f f a f c ea e HBV a d f ec a a HBV i fec f. T g e a e e i g ea e i de ce a a a e T 1 ce a d CT f e e e HBV a d e a cia ed a i ja c i e (IFN γ , TNF α a d IL 2) de e ed a a a e f e i f e d f a f a j fec i [6,7]. O i a f a cci e effecie e c d b a c i e ed b e e f a f f a e a i c c d e a ce acci e c g a e f f e dg [8]. Cff e , a i c d (A) a e e acci e ad a ice ed b e F d a d D Ad i f a i (FDA) f e a , a d ed a a a i e e a i B acci e cff e c g c i i ed W i e a i a fe, i a e a i e e a ad a ice a e ed i b i a i e Mf e g, e A ad a ed i e e a i B acci e i a i d T 2 ad a ca effecie e a ce I G1 a i b d f e e, b i i g e a cia ed i T 1 e e e e e [9]. F g f e, A i f a i a ce edia ed e e e, a d a ac i e b c aci a i a d diffe e i a f f CD8⁺ CTL f

^{*} C_{ff} e d) a f.Te.: +86 571 8697 1091; fa : +86 571 8697 1091. *E-mail address:* @ .ed .c (H. X. S).

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F \equiv **1.** Ce ica f c f e f. a c d i D (PD, C₅₇H₉₂O₂₈, MW: 1224.5854) fef f *P. grandiflorum*. I f c f e a e cidaed e ba i f ce ica a d ecra e ide ce i c d i a ecre e_f a d cea a e ic f e a ce ecr c . A i, β D a i f f a ; A a, α L a ab i f a ; G c, β D c f a ; R a, α L f a f a ; X, β D f a .

1 1		a). Н	IB A	[10]. I	add 👔 🌶	, A	/ e		Ì
cca 🕻	a	ca / ead	: 1	a	e }e	f i ec i	а	da	e	a

2.6. Splenocyte proliferation assay

S. ee c ec ed f ... e HBA i ... i ed ice de a e. icc di i , i Ha baaced a i (HBSS, Si a), a iced i a ai f cirada ed rafie ee e bala ee ce el, ad eg ce ge ed la l. c / de (0.8%, /.). Afg ce / f a) $(380 \times g = 4^{\circ}C f_1 = 10^{\circ})$, e.e. e.e. e.e. e.e. e.e. e.e. a.e. e. ree i e i PBS, a dre le ded i cliee edil. Ce be gec ediae cegbiabede ec i ec i e. Ce jabil e ceeded 95%. Se c e ge ig ig eeded) jee fje fa 96 e fla b $a = (N c)a 5 \times 10^6 ce / 100 \mu c$ ee ed), ee af $q \in A$ (fiac ce $fa = 5 \mu / \beta$), LPS (fiac ce $fa = \beta$) $10\,\mu$ /), HBA (fiac ceral $4\,\mu$ /), red re added 11 afia $e f 200 \mu$. Te a e g e c ba e d a37°C1 a 1 da g e 1 5% CO₂. Af g 44 (f C A a d LPS) $_{1}^{\prime}$ 68 (f $_{1}^{\prime}$ HB A), 50 μ fMTT $_{1}^{\prime}$ (2 /) a added eac e a dìc ba ed ff ff e 4. T e a e ece f f ed (1400 × g,5.) a d e f a ff ed MTT a e ed ca ef b i e i 150 μ fa DMSO (Si a, USA) f i) (192 μ DMSO) 8 μ 1NHC) a added each e, a d e aby bace a e a a ed i a ELISAy ead q a 570 af q 15 i. Telal de (SI) a cacaedbaed ef fi a: SI = eab i ba ce a efi i e c i e di ided b eab f ba ce a eff l aedc re.

2.7. Measurement of HBsAg-specific antibody

HBA ecific IG, IG1, IG2a, a dIG2ba ibdie je ea qedeeced) i di ida qaa e bai di ec ELISA. I by lef, log log are greated 1 100 μ HBA 1 (2 μ / 1 50. M carb are blocarb are b ffg, H 9.6) fg 24. a 4°C. T. e e e e a ed e e e PBSc aj) 0.05% (./.) T ee 20 (PBS/T ee), a d e b c ed) 5% FCS/PBS a 37 °C fr 2. Af φ ree a i, 100 μ fa φ ref d) ed φ a a er 0.5% FCS/PBS a cr φ eadded rice e . The aer φ ere ic baed fr 2 a 37 °C, f edb ree refreder ($C = 1^{\circ}$ ($C = 1^{\circ}$) c baed fy 2 a 37 °C. Af q a), e q) dae ac) j a a aed a f): 100 μ f by a e) (10 f O_{1} e edja jead 37.5 μ f 30% H₂O₂ j 25 f 0.1 M cly a e ... a e b ffe, H 5.0) a added eac e.T.e. a e a ic baed fy 10. i a 37°C, a de ereacia a g j a ed b add j 50 μ / e f 2N H₂SO₄. T e jca de ja eaged) a ELISA geader a 490, gee fea a e a e bee b ec ed la la d be ee la c .a), ELISA a a gelgfjed e aedafjaf ea.e.

2.8. Assay of natural killer (NK) cell activity

T e NK ce ac i f ee a de q i ed i a C 96R N Radi ac i e C i c i A a Ki (P e a). YAC 1 ce q e ed a q e ce a d eeded i 96 e U b iq iq a e a 4×10^4 ce i e i RPMI 1640. S e c e r e q ed a de q ibed ab e q e ed a eeffec r ce a d q e added a 2×10^6 ce i e i e E/T_r a i 50:1. Eac e a r e a ed f r e . T e a e a ce r if ed a $250 \times g$ f r

Tab e 1					
Se le ce	f.11 g	ed f <i>f f</i> ea) e	а	👔 a 👔 e RT PCR.

Ge e	Pjgeece	P, d, c }e/b.	Acc .
GAPDH	5 AAATGGTGAAGGTCGGTGTG 3 5 TGAAGGGGTCGTTGATGG 3	108	NM_001001303
IL 2	5 GCACCCACTTCAAGCTCCA 3 5 AAATTTGAAGGTGAGCATCCTG 3	174	NM_008366
IFN γ	5 CGGCACAGTCATTGAAAGCCTA 3 5 GTTGCTGATGGCCTGATTGTC 3	199	NM_008337

GAPDH, cora de de 3....a e de of e a e.

e (ABC). Af φ] c ba i f f 30 i, a e φ e a ed ad de e ed i er a e be id i e (TMB) a 37 ° C f f 15 i. Tereac i a ed b add i f 100 μ f i i. Teab i bace a eared i a ELISAr eader a 450 i.

2.11. Real-time RT-PCR for cytokine gene expression

Secefice ? e a da de gibed bef e geeded ? a 24 e fla b i g ? g a e (N c) a 5×10^6 ce / ? 1. c e e ed? , geaf g HBA (fi a c ce ga? 4μ /) a added ? ? a fi a e f 2 (g ? ca e e). T e a e ge? c ba ed a $37 \circ C$? a ddfied a ge? 5% CO_{2easu5425.per87.518.9n3(dasu5425.bit)7itiou5425.mix-306.u5425.213.9737 0 TD 05.4394}

760



F \equiv 3. Effect f. a c d) D (PD) HBA certific I G, I G1, I G2a, a d I G2b a jb d j HBA j jed jce. Set a grect ected 2 eet af grea j jaj, a d HBA certific I G, I G1, I G2a, a d I G2ba jb dje j e gra grectear jedba j djec ELISA a de g jbed j eet. Te a e grege e eda eat \pm S.E. (n=5). Sj jifica djiffgrecte j HBA a e j gre de j a eda ${}^{a}P < 0.05, {}^{b}P < 0.01, a d {}^{c}P < 0.001; e j HBA / A j a d <math>{}^{a}P < 0.05, {}^{e}P < 0.001, a d {}^{f}P < 0.001.$

IG1, IG2a, ad IG2ba (bd) qi equeque ea jed2 ee afque a jaj jELISA ad e / e ge i Fi.3. HBA a eid ced e e f HBA ec/fic IG, IG1, IG2a, a dIG2ba /bd. A a d PD j jfica e a ced e ç HBA ecific I G a d I G 1 1q] HB A]] ed ice (P < 0.05, P < 0.01, p < 0.001). Heg, ec blal fHBA / PDe ec/a ad e f 100 μ / d ced) ϕ ϕ a IGadIG1) ϕ c a ed) A a dHBA c b) a) S) fica e a ce e) HBA ecific q I G2aa dI G2b jq q e b q ed j PD j j ed j ce c q ed j HBA a e_{f} (P < 0.01 fP < 0.001). M₁ e q, I G2a a d I G2b a *j*b d *j* q *j* e *j* ce led l PD ge lg a el eA jeaed 1 ice. To ge ge, e.g., i ifica diffge ce (P > 0.05) i e ç IG2aa dIG2b e e be ee ice i i i ed *i* HBA/A a dHBA a e.T e fi d*i* d*i*ca ed a PD) /fica e a ced e HBA ec/fica /bd /dc / led i HBA.Meg, a ibd ig idced i ice i b PD ad a ed HB A af e } ec } e e cred b A ad a ed accie. ge i i ga

3.3. Effects of PD on NK cell activity in mice immunized with HBsAg

 Teeffec
 f PD
 NK ce ac i i i ice i ied

 i
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 q e
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 ac i i f NK ce i e HB A
 ifica
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F g-4. Effec f. a c d) D(PD) NK ce ac $i \neq j$ (ce) i d i HBA. S. e c e $g e_f e_f e_f e_d 2$ ee af $g = e_d i$ i = i, a da a ed f_f NK ce ac $i \neq j$ b $eLDH_f eea e$ e da de g bed i = e. T e a e $g e_f e$ e ed a ea $\pm S.E.$ (n=5). Sj fica differe ce i HBA a e_f g e de j a ed a $^cP < 0.001$; e j HBA /A f_f a $e^cP < 0.01$ a $d^fP < 0.001$.



F = 5. Effec f. a c d i D (PD) CTL ac i i i i ce i i ed i HBA. S. e c e q e f e q ed 2 ee af q e a i i a i a d a a ed f CTL ac i i b eLDH eae e d a de q i bed i e e. T e a e q e f e e d a ea \pm S.E. (n=5). Si if ica differe ce i HBA a e a d HBA /A q q e de i a ed a c q<0.001 a d f p<0.001 f e e c i e

d e (P < 0.001). T $q = q = e_1$, $p = q = f_1$, $p = f_1$, $q = f_2$, $p = f_1$, $q = f_2$, $p = f_1$, $p = f_2$, $p = f_1$, $p = f_2$, $p = f_1$, $p = f_2$, $p = f_2$, $p = f_2$, $p = f_1$, $p = f_2$, p

3.4. Effects of PD on specific CTL activity in mice immunized with HBsAg

Teeffec fPD a le ecific CTL ac li le HBA le le le ge l'Fl.5.1 la f. ice l HBA a e d ced e g ecific CTL ac li 2 ee af g e ec da la la Addil fA HBA did re lfregigeael HBA ecific CTL ac li ab e e ee l HBA a e.I c ra , PD lifica e a ced e ecific li ac li fCTL ice led l HBA a ree d e (P<0.001). Tefi d lidica ed a PD c d.r e ecific li ac li fCTL ice led l HBA.

3.5. Effect of PD on cytokine secretion by splenocytes from HBsAg-immunized mice

I į dę a e effec f PD T 1 a d T 2 c i e re d∤ HBA, c] e / d c] f . e c e] led ice gedeeced lELISA. Te calipa HBA 👔) cje fIL 2, IFN γ, a dIL 10 gec j ced). e c le adad, ad ej crrea l cefficie geable a 0.9980. A *j* Fj. 6, ec e fc *j* e IL 2, IFN γ, ad IL 10 / ec /e gaa f HBA / aed ecefie (ce) i de l'HBA/PD ge) i q a e i HBA c f i ce (P < 0.01),a PD / fica e a ced e / d c / f e e i Tlad T2 c / e / HBA / / ed /ce. H e g, A) jfica) ç ea ed eIL 10 / d c i eHBA *i* ed *i*ce (*P*<0.001). Ì

3.6. Effect of PD on mRNA expression of cytokines in splenocytes from HBsAg-immunized mice

Sice PD i fica e a ced I G2a a d I G2b a ib d re. e a dT 1 c i e d ci, e e ea red IL 2 a dIFN y RNAe re i i e c e f ice i ied i HB A T e e c e f ei ied ice ge i a ed i i i HB A, a d a RNA gee raced. Rea i e a ia ie RT PCR fr IL 2 a d IFN y c i e RNAe re i ge g fr ed. GAPDH a eda a raia i c fr e RT PCR reac i A i Fi.7, e IL 2 a d IFN y RNAe re i i e ice i ied i PD ge i g a





F \equiv 7. Effect f. a c d D (PD) RNA e fe f fc fe IL 2 a d IFN γ i e c e f e HBA f fed ice. S e c e ge fe a ed 2 ee af g e a f fa , a d c fed f HBA (fi a c ce fa 1 4 μ /) ff 24. T e RNA e fe f e fGAPDH, IL 2, a d IFN γ ge deeced b fea fer PCR fed for for for a left for a ce fe for a ce fe for a left for a ce fe for a left for a ce fe for a ce fe

e) eHBA a eadHBA/A f (P < 0.001). T q e q e, e q, j jfica djff q e ce (P > 0.05) j e IL 2 a d IFN γ RNA e f e j be ee jce f j ed j HBA/A ad HBA a e. T, fi dj f f q c fi ed a PD j jfica j d ced T 1 c j e eq e j b e c e f e HBA j j ed jce.

4. D. c. .. 🕅

E ide ce e i cea e a T 1 / T 2 e e, e g a ed a i e ic i a i , ca be d a ed i i de e di e ad a ed f i i a i [15,16]. T e diffg e T 1 a d T 2 i e e e fie crre d e acia i f di c a be f T ce c a a c g i ed b ei a g f c i e d c i [17]. T 1 e e a e a cia ed i IFN y, IL 2 a d IL 12 y d c i a de a ce i b i e c i c i I G2a, I G2b a d I G3. T e T 1 y e e ca be crre a ed i e i d c i f ce edia ed i [18], ic a bee de g ibed a bei i i ca ed f e e i a i f i d i i fec i [19,20]. T 2 y e e, ic c r e a a d diffg e ia i [21], a e c a a c i ed b i e e f ci c a i a ib die, f a i I G i e, a d e e g e f e c i e IL 4, IL 5, IL 10 a d IL 13. Fy geclel l cg al lfecl deae diffge jald cl fT 1 g T 2 ge e b j ge jed.

A i e e a i e e a i B accie c e c e cia i ed. A ad y bed accie a e bee e icie e ia T 2 i ere e, a d j d ce ce edia ed i i. I a re red a a T 2 ad a , c a A , i efficaci i e c e i i i re di a a , i e a ad a i d ci a baa ced T 1/T 2 re e a ai HB A a abe ec e re i e HB A i B10.M ice. Mea i e, ce a de jaed a PD daed e aj fj. eje. e, a dejojedabaa oed T1/T2] eje. e HBA] joe a a ojaed e jje jae a oe e fIG2a, IG2bad IG1 e e [29].

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