

(19)(12)(KR)(A)

(51) 。 Int. Cl.⁷
A61K 39/245

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(43)

10-2004-0074190
2004 08 23

(21)10-2003-0009875

(22)2003 02 17

(71)

431403

31

134

223-23

(72)

101-601

6 APT111-602

110-4 APT1-505

가 1 115-84201

4 2 206-901

756 ()1-1103

2 3

(74)

:

(54)DNA

85ADNA , DNA DNA DNA DNA I D(gD) Ag

. DNA 가

4

1 pGX10-Ag85A COS7 Ag85A

2

A : 4 ,
B : 18 ,
C : 42

3

A (B) Ag85A (A) (C) (INH PZA) , DNA 30 IFN- CF(culture filtrate) DN

4

DNA
A : 30 ,
B : 30 ,
C : 42 ,
D : 42

DNA , DNA
1,000 1/3(19) (*Mycobacterium tuberculosis*) 800
HIV 가 , 300 ,
5000 가 HIV 90 가가
(multi-drug resistant tuberculosis; MDR-TB)

가 , 가 50% , 600 HIV/TB

가

가 5-10% (Kochi *et al.*, *Lancet* 1997, **350**: 142; Toossi and Ellner, Pathogenesis of tuberculosis. In: Friedman LN (ed). Tuberculosis: current concepts and treatment. C RC Press: New York, 2001, pp.19-47).

(Chemotherapy) BCG (Rieder HL, Internati onal Union Against Tuberculosis and Lung Disease: Paris, 2002). DOTS (directly observed therapy, short course) 85%

, 6 가 , 가

12% (Broekmans JE, Control strategies and program me management. In: Porter JDH, McAdam KPWJ (ed). Tuberculosis: Back to the Future. John Wiley and Son s: New York, 1994, pp. 171-192; Chaulk *et al.*, *J Am Med Assoc*, 1998, **279**: 943-948; Mitchison DA, *Tuber cle*, 1985, **66**: 219-225).

BCG (*Mycobacterium bovis*)

가 M. (*M. tuberculosis*)

DNA M.

DNA 85A, 85B, 65 kDa heat shock protein PstS-3 (Denis O *et al.* . *Infect Immun* 1998; **66**: 1527-1533; Huygen K *et al.* . *Nat Med* 1996; **2**: 893-898; Lozes E *et al.* . *Vaccine* 19 97; **15**: 830-833; Kamath AT *et al.* . *Infect Immun* 1999; **67**: 1702-1707; Tascon RE *et al.* . *Nat Med* 1996; **2**: 888-892; Lowrie DB *et al.* . *Vaccine* 1997; **15**: 834-838; Tanghe A *et al.* . *J Immunol* 1999; **162**: 1113-1119).

, DNA (anti-mycobacterial immunity)

CTL(cytotoxic T lymphocyte) Th1 (Bonato VL *et al.* ., *Infect Immun*, 1998, **66**: 169-175).

M. (M. vaccae) IFN- IL-12 M. (Cornell model) (C orlan E *et al.*, *Respir Med*, 1997, **91**: 21-29; Holland SM, Dorman SE., *J Invest Med*, 1998, **46** (Suppl.) 218A; Holland SM *et al.* ., *N Engl J Med*, 1994, **330**: 1348-1355).

DNA (Turner J. *et al.* ., *Infect. Immun.*, 2000, **68** : 1706-1709; Repique CJ *et al.* ., *Infect. Immun.*, 2002, **70** : 3318-3323). , hsp65 DNA (Lowrie DB *et al.* ., *Vaccine*, 2000, **18**: 1712-1716; Lowrie DB *et al.* ., *Nature*, 1999, **400**: 269-271).

M. (macrophages) T (Andersen P, *Trends Immunol* 2001; **22**: 160-168). CD4+ CD8+ T (Boom *et al.*, *Infect Immun*, 1991, **59**: 2737-2743; Kaufmann SH., *A nnu Rev Immunol*, 1993, **11**: 129-163; Tan JS *et al.* ., *J Immunol*, 1997, **159**: 290-297).

IFN- - CD8+/CD4-/CD44^{high} CTL (Bonato VL *et al.* ., *Infect Immun* , 199 8, **66**: 169-175).

가 (adjunct)가

IL-12 M. (M. avium) (Leishmania (Nabors *et al.* ., *Proc Natl Acad S*

IL-12 DNA Ag85 - IFN- 가 가
T- IL-12 DNA 가 Th1 .
M.

3 INH PZA Ag85A DNA IL-12 DNA DNA INH PZA
M. M. 30 DNA IL-12 DNA A B).
80% 100% , IL-12 DNA 4 4 B).
40%가 Th1 , IL-12N22
OL DNA M. IL-12 DNA Th1
M. IL-12N220L DNA Th1
A 4 B).
DNA (adjuvant) , INH PZA 20% M. Ag85A IL-12 (4
M.

DNA M. , INH PZA 80% 60% M.
42 가 (4 C 4 D , 1). DNA 60%
가 , IL-12 DNA M.
IL-12 DNA가
(Ha SJ *et al.*, *Nat Biotechnol*, 2002, **20**: 381-386). 30
, Ag85A DNA 20% M.

DNA
DNA
(vehicle)
-N -N (2- - , N,N-
IC, ; MPLR(3-O- A; RIBI
ImmunoChem Research, Inc., Hamilton, Montana);
(cochleates),
ISCOMS(
DNA
mg 1 0.02 0.1 mg 1 kg DNA 0.1 0.2

< 1> DNA

<1 - 1> Ag85A DNA

DNA 1
 I D(gD) 2 Ag85A
 pTV2 (Lee et al., *J Virol* 1998; **72**:
 8430-8436) 'pGX10'
 'pGX10-Ag85A'
 pGX10 2003 2 3 (: KCTC 10418BP).
 Ag85A 가 , pGX10-Ag85A DNA COS7
 -Ag85A
 Ag85A
 , Ag85A 가 (empty vector) pGX10 Ag85A
 pGX10-Ag85A Ag85A
 가 (1).

<1-2> IL-12 DNA

IL-12 p40 Asn-222 Leu-222 IL-12 IL-12p
 40 가 (Ha SJ *et al.*, *Nat Biotechnol*, 2002, **20**: 381-386), HCV(hepatitis C v
 irus) E2 DNA IL-12 DNA IL-12 DNA E2- CTL
 IFN- - CD8+ T (Ha SJ *et al.*, *Nat Biotechnol*, 2002, **20**: 38
 1-386). , DNA IL-12 DNA DNA (construct)
 , IL-12 DNA IL-12p35(mp35),
 (encephalomyocarditis virus; EMCV) IRES(internal ribosomal entry site)
 IL-12p40(mp40) cDNA <1-1> pGX10
 'pGX10-mp35/IRES/mp40(pGX10-IL-12)'
 IL-12 DNA 가 IL-12p40(mp40) Asn-220 N-
 , PCR Asn-220 Leu-220 (Holland SM *et al.*, *N E
 ngl J Med*, 1994, **330**: 1348-1355). , pGX10-IL-12 mp40 Asn-220 Leu-220
 IL-12 DNA , 'pGX10-mp35/IRES/mp40-N220L(pGX10-IL-12N220L)'

< 2> (Latent infection model)

<2-1>

(M. tuberculosis)
 (Sauton medium) (pe
 llicles) 10 , M H37Rv(ATCC 27294) 3 mm
 -70
 (Middlebrook) 7H11 가(Difco, Detroit, Mich.) M.
 PBS 250,00
 0 pfu/M ℓ

<2-2>

(Cornell model)(McCune et al., *J Exp Med* 1966; **123**: 445-468)
 , 5-6 C57BL/6J (SLC , Shizuoka, Japan)
 (Glas-Col, Terre Haute, Ind.) <2-1> M. H37
 Rv 250,000 pfu/M ℓ 10 M ℓ 60
 M.
 , 0.05% Tween 90
 100 $\mu\ell$ M7H11 가 (Difco, Detroit, Mich.)
 M.
 log₁₀ CFU(colony forming unit)/

200 M. 가

M. 가
 , M. 4 10%
 , 2 3 μ m
 (haematoxylin) (eosin)

(monocytes) (2 A),
 , 6.69 \pm 0.20 cfu(log₁₀) 5.17 \pm 0.11 cfu(log₁₀)

<2-3>

<2-2>

<2-2> 4 25 mg/kg
 /day (isoniazid, 'INH') (Sigma-Aldrich, Co., MO, USA) 1,000 mg/kg/day
 (pyrazinamide, 'PZA') (Sigma-Aldrich, Co., MO, USA) 가 3
 . INH PZA <2
 -2>

, INH PZA 3 2 (18),
 (2 B), M.
 . 42 , INH PZA 26 (2 C)
 . 30 5 4 (80%) 5 (100%)
 M. 가
 (1).
 (McCune et al., *J Exp Med* 1966; **123**: 445-468). , INH PZA 6
 30 42 가 (1).

[1]

	30	42		30	42	
INH + PZA (6)	0/5 ^a	0/5	0/10	0/5	0/5	0/10
INH + PZA (3)	4/5	4/5	8/10	5/5	3/5	8/10
INH + PZA (3)+ DNA	3/5	3/5	6/10	3/5	3/5	6/10
INH+PZA (3)+IL-12N220L DNA	1/5	2/5	3/10	2/5	2/5	4/10
INH + PZA (3)+ Ag85A DNA	0/5	1/5	1/10	1/5	1/5	2/10

a : M. / H37Rv M.

< 3> IL-12N220L Ag85A DNA - IFN-

DNA 가
 4 INH PZA 3
 pGX10, pGX10-IL-12N220L pGX10-Ag85A DNA 50
 18 , 30 , 42 , 2 , 1
 (3 A).

30 CF(J.T. Belisle, Colorado State Universit
 y, Fort Collins, Colo.) Ag85A (Standardia Inc., Suwon, Korea)
 IFN- . IFN- .

, 2×10^5 200 μ l RPMI 10 μ g/ml CF Ag85A 6
 gen, San Diego, Calif.) IFN- IFN- OptEIA™ (Pharmin
 , INH PZA CF Ag85A IFN- 가 INH PZA
 PZA CF Ag85A CF IFN- 가 DNA INH
 Th1 INH PZA IFN- (3 B 3 C),
 가 IFN- 가
 (Dieli F *et al.*, *Scand J Immunol*, 2000, **52**: 96-102; Wilkinson RJ *et al.*, *J Infect Dis*, 1998, **178**:
 760-768). DNA(pGX10) CF
 Ag85A IFN- (3 B 3 C),
 DNA CpG 가 Th1 (Krieg AM. *V*
accine 2000; **19**: 618-622). IL- 12N220L DNA DNA Ag85A DNA
 CF IFN- (3 B , p<0.03 p<0.04). Ag8
 5A DNA - CF IFN- (3 B), DNA
 IL-12N220L DNA - Ag85A Th1 Ag85A DNA
 C , p<0.01 p<0.04). IL-12N220L DNA Ag85- IFN-
 가 가 (3 C , p<0.04), DNA - T- IL-1
 2N220L DNA Th1 -

< 4> IL-12N220L DNA Ag85A DNA

<4-1> DNA

IL-12N220L DNA Ag85A DNA
 30 , 3 INH PZA 가 14
 (4 A 4 B).
 , DNA INH PZA (80% 100%) 가
 M. 가 DNA(pGX10) 5 CpG 가 Th1 3 (60%)
 M.
 , 3 INH PZA IL-12N220L DNA , 5
 1 (20%) 2 (40%) M. 가 (4 A 4
 B , 1) IL-12N220L DNA Th1 (Ha SJ *et al.*,
Nat Biotechnol, 2002, **20**: 381-386). , IL-12N220L M.
 Th1 M.
 가
 , Ag85A DNA 5 M. 가 , 5
 1 (20%) IL-12N220L Ag85A (4 A 4 B , 1).
 INH PZA M. DNA
 M. (adjuvant)

<4-2> DNA

M. DNA ,
 42 , 3 INH PZA 가 26
 , INH PZA 5 4 (80%) 3 (60%)
 M. 가 , DNA가 60%
 , IL-12N220L DNA 5 2 (40%) M. 가
 (1). IL-12N220L DNA가

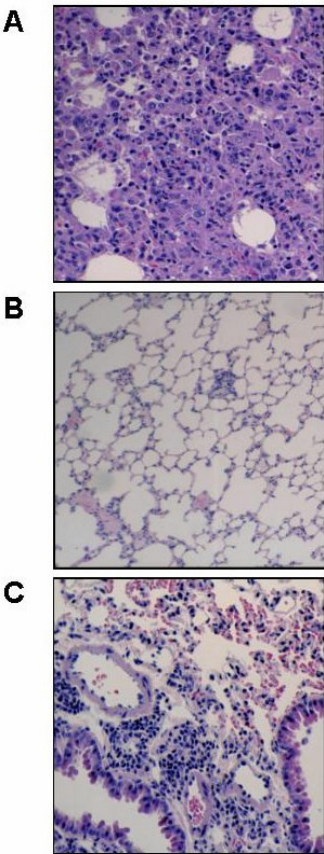
(Ha SJ *et al.* . *Nat Biotechnol* 2002; **20**: 381-386).

30 Ag85A DNA 5 1 (20%)
M. (4 C, 4 D 1). , IL - 12N220
L DNA (p<0.05, Chi-square) Ag85A DNA (p<0.01, Chi-square)
M. . Ag85A DNA
(p<0.05, Chi-square), IL - 12N220L DNA
가 Ag85A- IFN- 가
, CF- IFN- . Ag85A- Th1
M. .

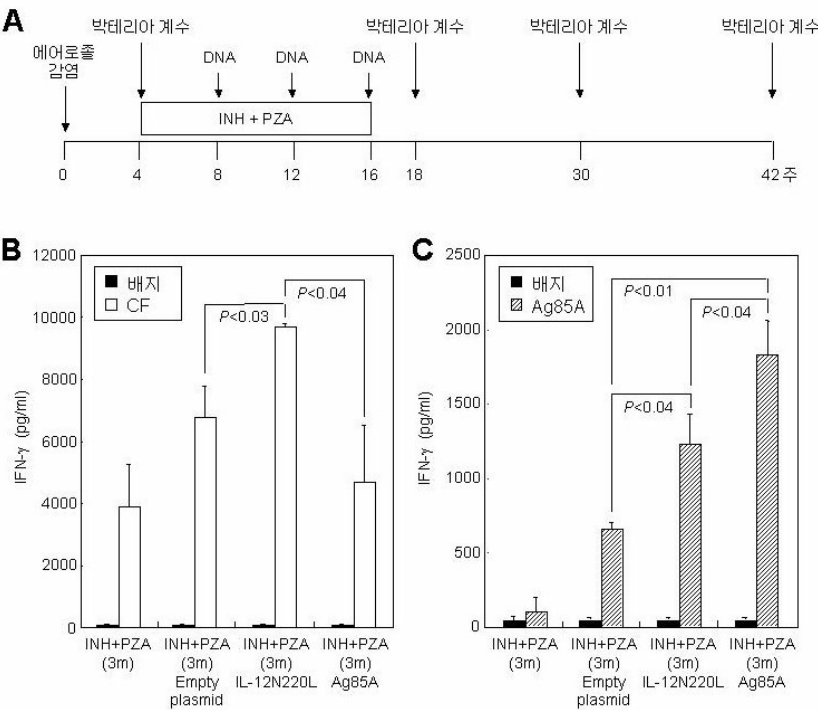
가 DNA 가 I D(gD) Ag85A
가 , 가
가 , .

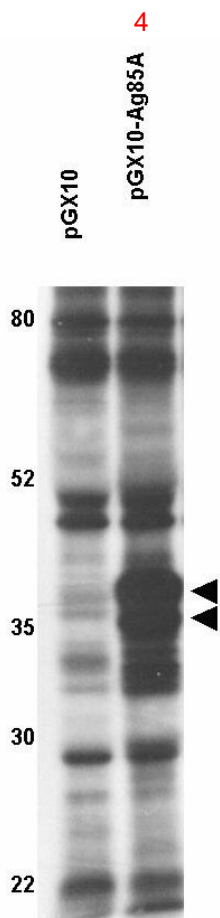
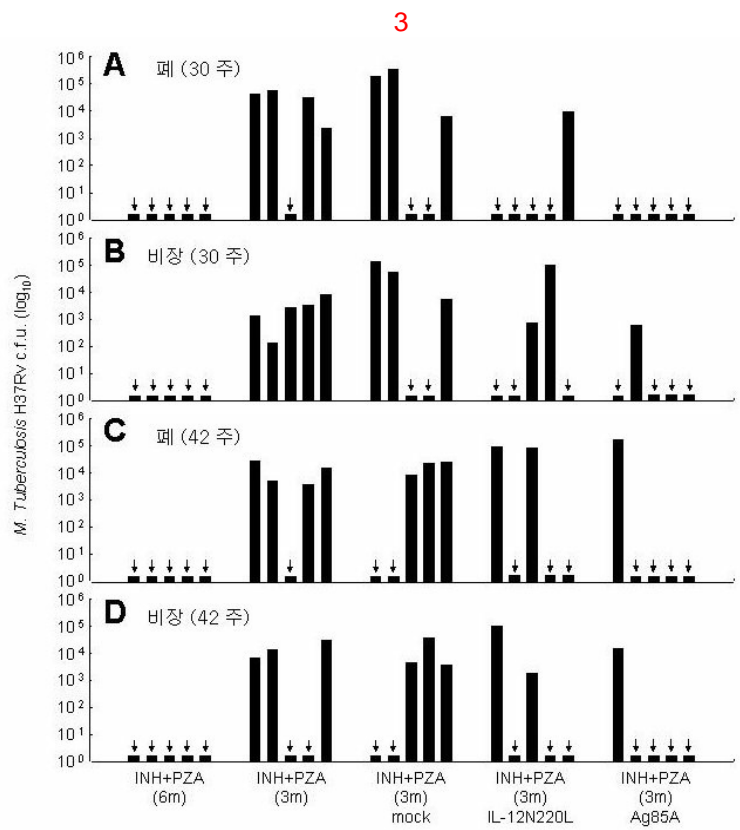
- (57)
1. I D(gD) Ag85A DNA .
 2. 1 , DNA I D(gD) 1
DNA .
 3. 1 , Ag85A 2 DNA .
 4. 1 , 1 Ag85A 가 pGX10 pGX10 - Ag85A I D(gD) DNA (; KCTC 10418BP).
 5. 1 4 DNA .
 6. 5 eptomycin) , (INH), (PZA), (rifampin), (str
(ethambutol) .
 7. 6 , .

1



2





<120> Therapeutic DNA vaccine for tuberculosis and pharmaceutical
composition containing the same

<130> 2p-11-29B

<160> 2

<170> KopatentIn 1.71

<210> 1

<211> 60

<212> DNA

<213> herpes simplex virus 7

<400> 1

atgagggggg ctgccgccag gttgggggcc gtgattttgt ttgtcgtcat agtgggcctc 60

<210> 2

<211> 1017

<212> DNA

<213> Homo sapiens

<400> 2

atgcagcttg ttgacagggt tcgtggcgcc gtcacgggta tgtcgcgtcg actcgtggtc 60

ggggccgtcg gcgcggccct agtgtcgggt ctggtcggcg ccgtcgggtg cacggcgacc 120

gcgggggcat tttcccgcc gggcttgccg gtggagtacc tgcaggtgcc gtcgccgtcg 180

atgggccgtg acatcaaggt ccaattcaa agtgggtggg ccaactcgcc cgccctgtac 240

ctgctcgacg gcctgcgcgc gcaggacgac ttcagcggct gggacatcaa caccgcggcg 300

ttcgagtggg acgaccagtc gggcctgtcg gtggatcatgc cggtaggtgg ccagtcaagc 360

ttctactccg actggtacca gcccgcctgc ggcaaggccg gttgccagac ttacaagtgg 420

gagaccttcc tgaccagcga gctgccgggg tggctgcagg ccaacaggca cgtcaagccc 480

accggaagcg ccgtcgtcgg tctttcgatg gctgcttctt cggcgtgac gctggcgatc 540

tatcaccccc agcagttcgt ctacgcggga gcgatgtcgg gcctgttgga cccctccag 600

gcgatgggtc ccacctgat cggcctggcg atgggtgacg ctggcggcta caaggcctcc 660

gacatgtggg gcccgaagga ggaccggcg tggcagcgca acgaccgct gttgaacgtc 720

gggaagctga tcgccaacaa caccgcgtc tgggtgtact gcggcaacgg caagccgtcg 780

gatctgggtg gcaacaacct gccggccaag ttctctgagg gcttcgtgcg gaccagcaac 840

atcaagttcc aagacgccta caacgccgtt ggcggccaca acggcgtgtt cgacttcccg 900

gacagcggta cgcacagctg ggagtactgg ggcgcgcagc tcaacgctat gaagcccagc 960

ctgcaacggg cactgggtgc cacgccaac accgggcccg cgcccaggg cgcctag 1017