US 20140154288A1

(19) **United States**

(12) **Patent Application Publication**

Leclerc et al.

(10) **Pub. No.: US 2014/0154288 A1**

(43) **Pub. Date: Jun. 5, 2014**

(54) **PAPAYA MOSAIC VIRUS COMPOSITIONS AND USES THEREOF FOR STIMULATION OF THE INNATE IMMUNE RESPONSE**

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(21) Appl. No.: **14/117,363**

(22) PCT Filed: **May 1, 2012**

(86) PCT No.: **PCT/CA2012/050278**

§ 371 (c)(1),
(2), (4) Date: **Jan. 9, 2014**

Related U.S. Application Data

(60) Provisional application No. 61/485,955, filed on May 13, 2011.

Publication Classification

(51) **Int. Cl.**
A61K 39/12 (2006.01)

(52) **U.S. Cl.**
CPC ***A61K 39/12*** (2013.01)
USPC **424/204.1**

(57) **ABSTRACT**

The use of compositions comprising a papaya mosaic virus (PapMV) moiety for stimulation of the innate immune response is provided. The PapMV moiety may be papaya mosaic virus or PapMV virus-like particles (VLPs). The PapMV compositions stimulate a sufficiently strong innate immune response to provide protection against subsequent pathogen challenge or to treat an established infection. The use of PapMV compositions to protect a subject from potential infection by a pathogen, such as a viral pathogen, a bacterial pathogen or a fungal pathogen, and the use of PapMV compositions to treat an established infection, are also provided. The PapMV compositions be administered, for example, via intranasal or pulmonary routes to elicit effects within the mucosa and/or in the respiratory system.

A.

MSKSSMSTPNIAFPAITQEQMSSIKVDPTSNLLPSQEQLKSVSTLMVAAKVPAASVTT
VALELVNFCYDNGSSAYTTVTGPSSIPEISLAQLASIVKASGTSLRKFCRYFAPIIWNL
RTDKMAPANWEASGYKPSAKFAAFDFFDGVENPAAMQPPSGLIRSPTQEERIANATN
KQVHLFQAAAQDNNFTSNSAFITKGQISGSTPTIQF LPPPE

B.

ATGTCTAAGTCAAGTATGTCCACACCCAACATAGCCTTCCCCGCCATCACCCAGG
AACAGATGAGCTCGATTAAGGTCGATCCAACGTCCAATCTTCTGCCCTCCCAAGA
GCAGTTAAAGTCAGTGTCCACCCTCATGGTAGCTGCTAAGGTTCCAGCAGCCAGT
GTTACAACTGTGGCATTGGAGTTGGTCAACTTCTGCTATGACAATGGGTCCAGCG
CGTACACCACAGTGACTGGCCCATCATCAATACCGGAGATATCACTGGCACAATT
GGCTAGTATTGTCAAAGCTTCCGGCACTTCCCTTAGAAAATTCTGCCGGTACTTC
GCGCCAATAATCTGGAATCTGAGGACGGACAAAATGGCTCCTGCCAATTGGGAG
GCTTCAGGATACAAGCCAAGCGCCAAATTTGCCGCGTTCGACTTCTTCGACGGGG
TGGAGAATCCGGCGGCCATGCAACCCCCTTCGGGACTAATCAGGTCGCCGACCC
AGGAAGAGCGGATTGCCAATGCTACCAACAAACAGGTGCATCTCTTCCAAGCCG
CGGCACAGGACAACAACTTTACCAGCAACTCCGCCTTCATCACCAAAGGCCAAA
TTTCTGGGTCAACCCCAACCATCCAATTCCTTCCACCCCCCGAATAA

FIGURE 1

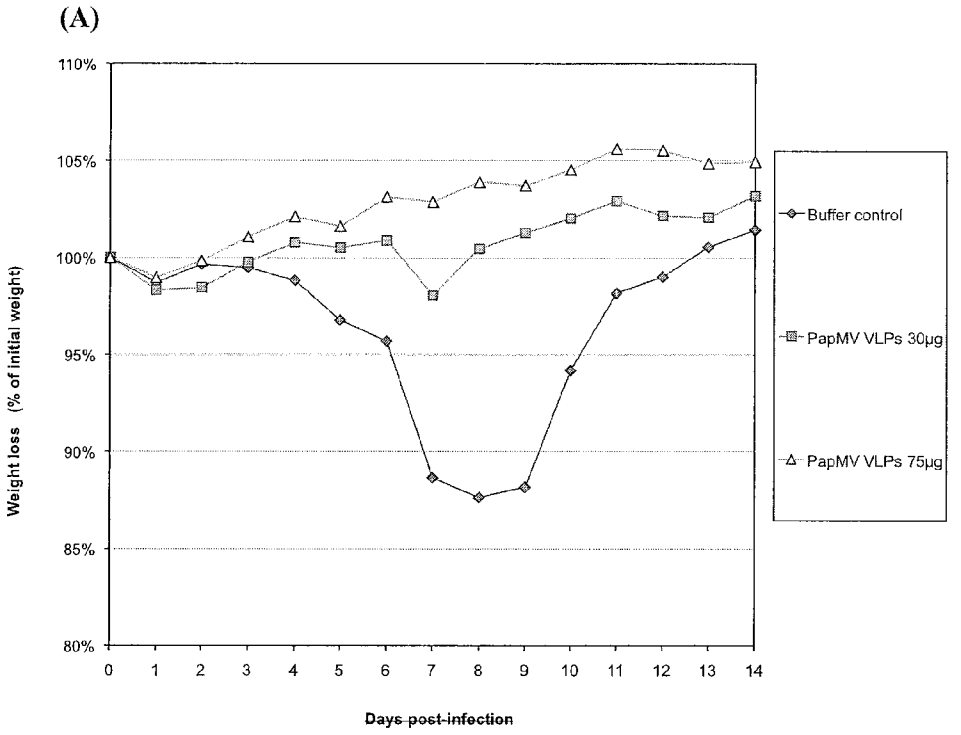
A.

MASTPNIAFPAITQEQMSSIKVDPTSNLLPSQEQLKSVSTLMVAAKVPAASVTTVALE
LVNFCYDNGSSAYTTVTGPSSIPEISLAQLASIVKASGTSLRKFCRYFAPIIWNLRTDK
MAPANWEASGYKPSAKFAAFDFFDGVENPAAMQPPSGLTRSPTQEERIANATNKQV
HLFQAAAQDNNFASNSAFITKGQISGSTPTIQFLPPPE

B.

MASTPNIAFPAITQEQMSSIKVDPTSNLLPSQEQLKSVSTLMVAAKVPAASVTTVALE
LVNFCYDNGSSAYTTVTGPSSIPEISLAQLASIVKASGTSLRKFCRYFAPIIWNLRTDK
MAPANWEASGYKPSAKFAAFDFFDGVENPAAMQPPSGLTRSPTQEERIANATNKQV
HLFQAAAQDNNFASNSAFITKGQISGSTPTIQFLPPPETSTTRHHHHHH

FIGURE 2


(A)
110%
105%
100%
95%
90%
85%
80%
Weight loss (% of initial weight)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days post-infection
Buffer control
PapMV VLPs 30µg
PapMV VLPs 75µg

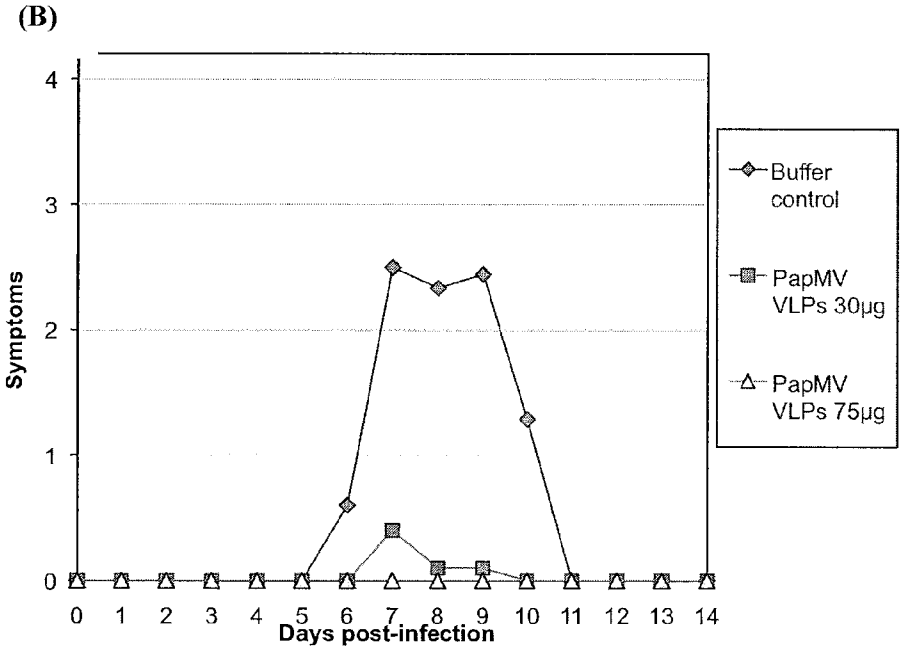

(B)
4
3
2
1
0
Symptoms
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days post-infection
Buffer control
PapMV VLPs 30µg
PapMV VLPs 75µg

FIGURE 3

(C)

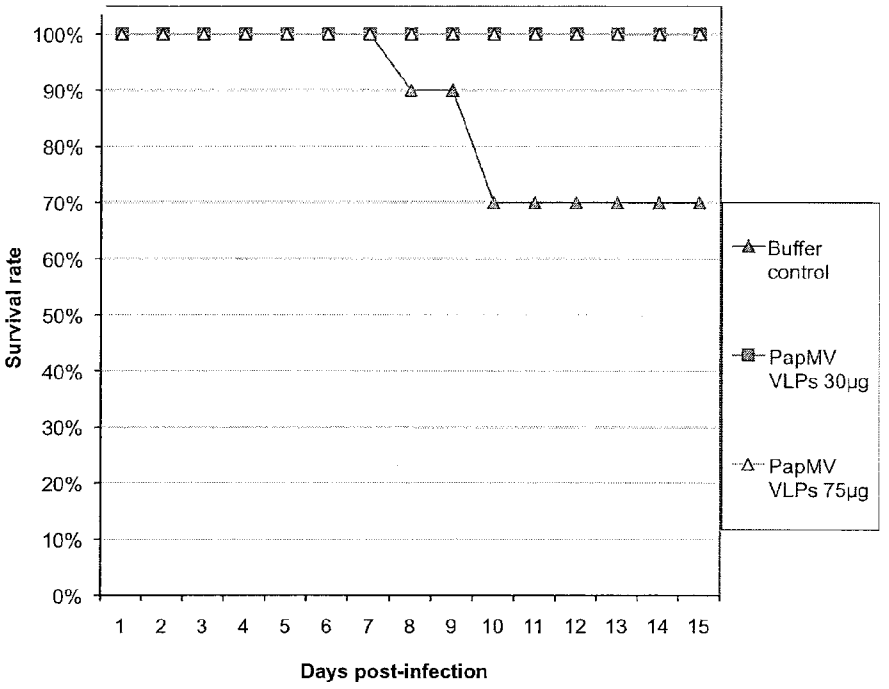
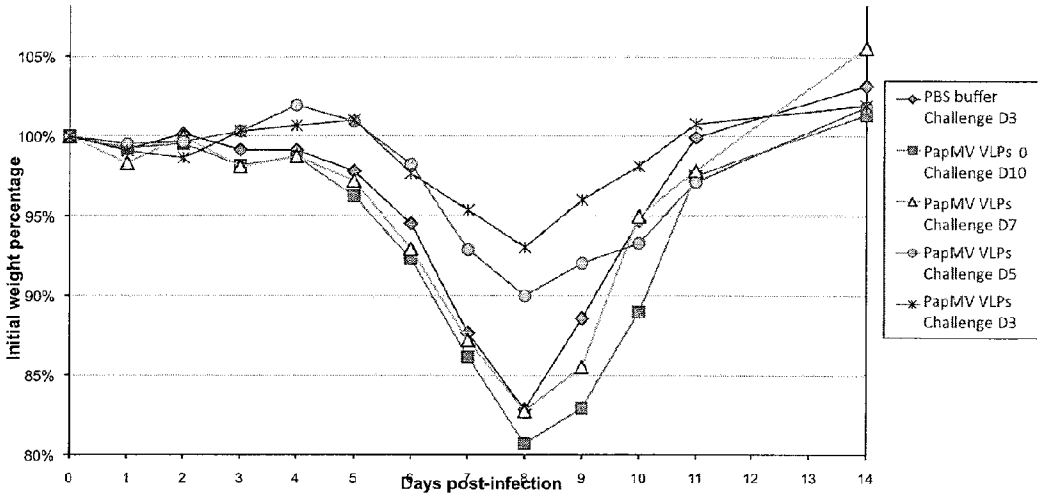


FIGURE 3 (con.)

(A)


105%
100%
95%
90%
85%
80%
Initial weight percentage
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days post-infection
PBS buffer Challenge D3
PapMV VLPs 0 Challenge D10
PapMV VLPs Challenge D7
PapMV VLPs Challenge D5
PapMV VLPs Challenge D3

(B)

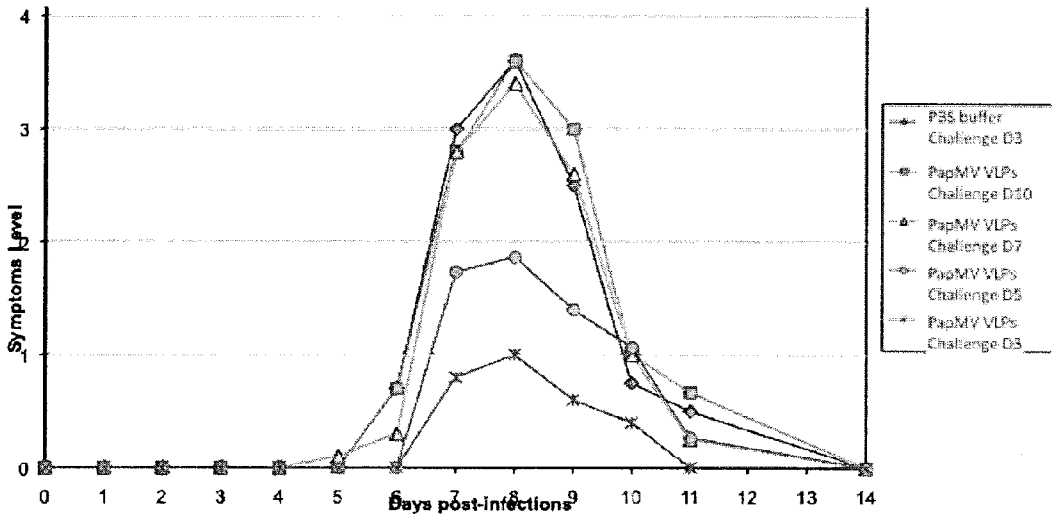

4
3
2
1
0
Symptoms Level
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days post-infections
PBS buffer Challenge D3
PapMV VLPs Challenge D10
PapMV VLPs Challenge D7
PapMV VLPs Challenge D5
PapMV VLPs Challenge D3

FIGURE 4

(C)

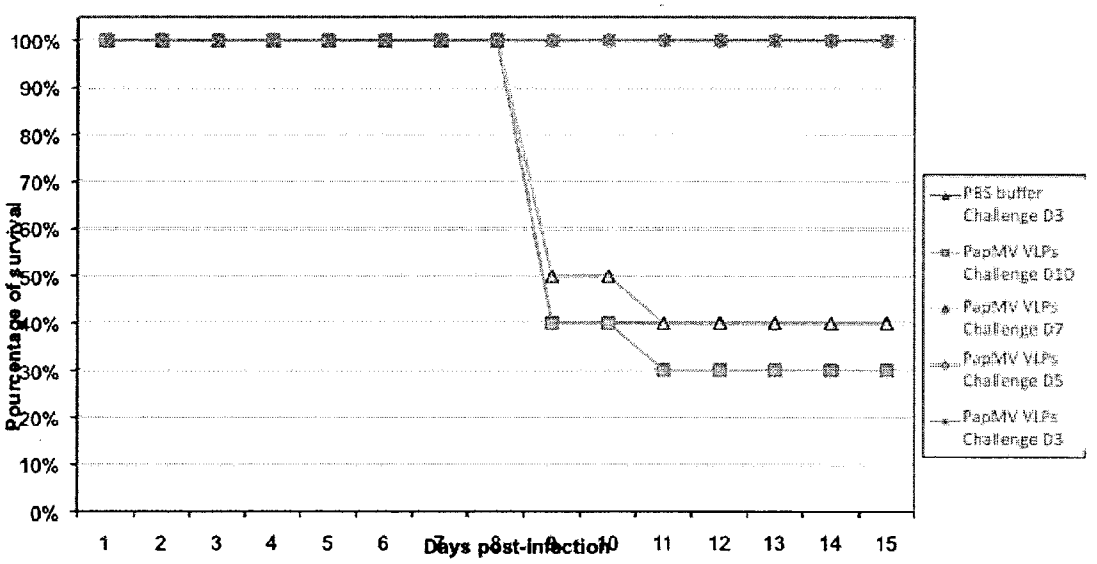
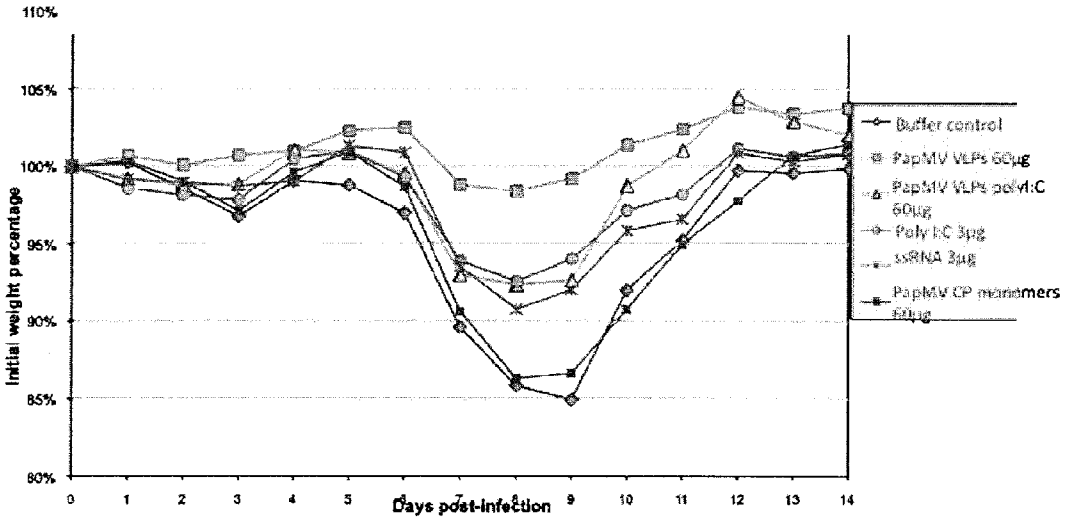


FIGURE 4 (con.)

(A)


110%
105%
100%
95%
90%
85%
80%
Initial weight percentage
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days post-infection
Buffer control
PapMV VLPs 60µg
PapMV VLPs polyI:C 60µg
Poly I:C 3µg
ssRNA 3µg
PapMV CP monomers 60µg

(B)

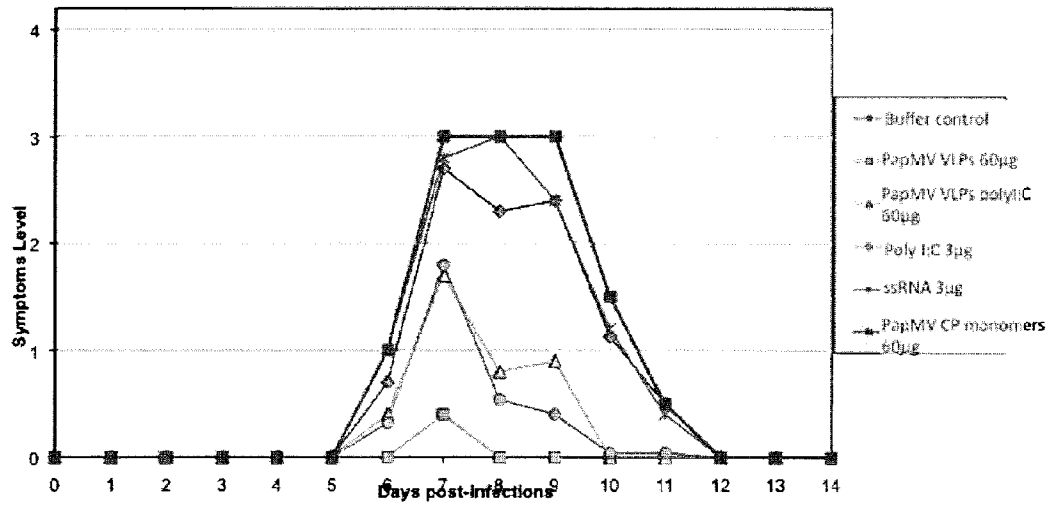

4
3
2
1
0
Symptoms Level
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days post-infections
Buffer control
PapMV VLPs 60µg
PapMV VLPs polyI:C 60µg
Poly I:C 3µg
ssRNA 3µg
PapMV CP monomers 60µg

FIGURE 5

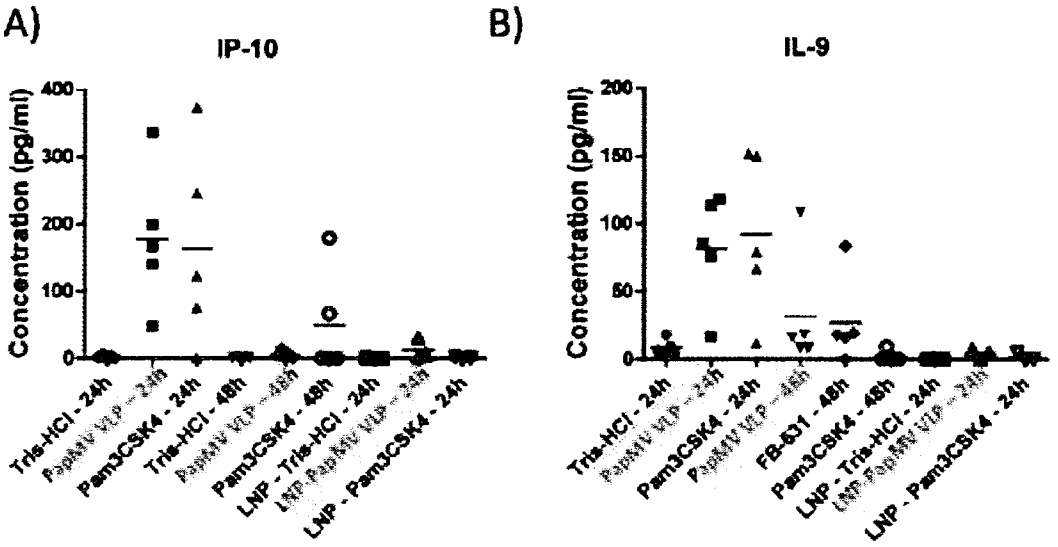

A)
IP-10
Concentration (pg/ml)
400
300
200
100
0
Tris-HCl - 24h
PapMV VLP - 24h
Pam3CSK4 - 24h
Tris-HCl - 48h
PapMV VLP - 48h
Pam3CSK4 - 48h
LNP - Tris-HCl - 24h
LNP PapMV VLP - 24h
LNP - Pam3CSK4 - 24h
B)
IL-9
Concentration (pg/ml)
200
150
100
50
0
Tris-HCl - 24h
PapMV VLP - 24h
Pam3CSK4 - 24h
PapMV VLP - 48h
FB-631 - 48h
Pam3CSK4 - 48h
LNP - Tris-HCl - 24h
LNP PapMV VLP - 24h
LNP - Pam3CSK4 - 24h

FIGURE 6

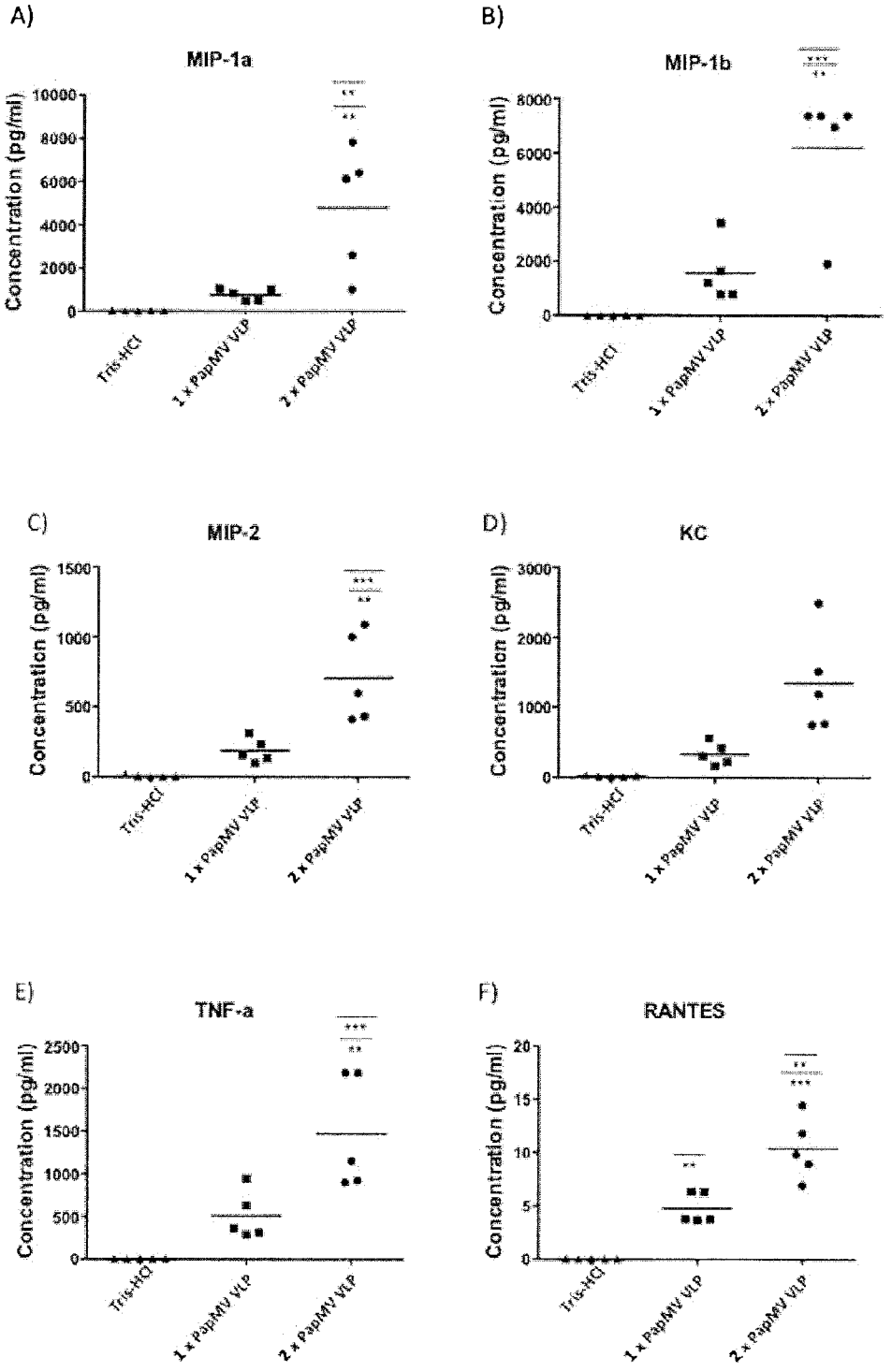

A)
MIP-1a
Concentration (pg/ml)
10000
8000
6000
4000
2000
0
Tris-HCl
1 x PapMV VLP
2 x PapMV VLP
B)
MIP-1b
Concentration (pg/ml)
8000
6000
4000
2000
0
Tris-HCl
1 x PapMV VLP
2 x PapMV VLP
C)
MIP-2
Concentration (pg/ml)
1500
1000
500
0
Tris-HCl
1 x PapMV VLP
2 x PapMV VLP
D)
KC
Concentration (pg/ml)
3000
2000
1000
0
Tris-HCl
1 x PapMV VLP
2 x PapMV VLP
E)
TNF-a
Concentration (pg/ml)
2500
2000
1500
1000
500
0
Tris-HCl
1 x PapMV VLP
2 x PapMV VLP
F)
RANTES
Concentration (pg/ml)
20
15
10
5
0
Tris-HCl
1 x PapMV VLP
2 x PapMV VLP

FIGURE 7

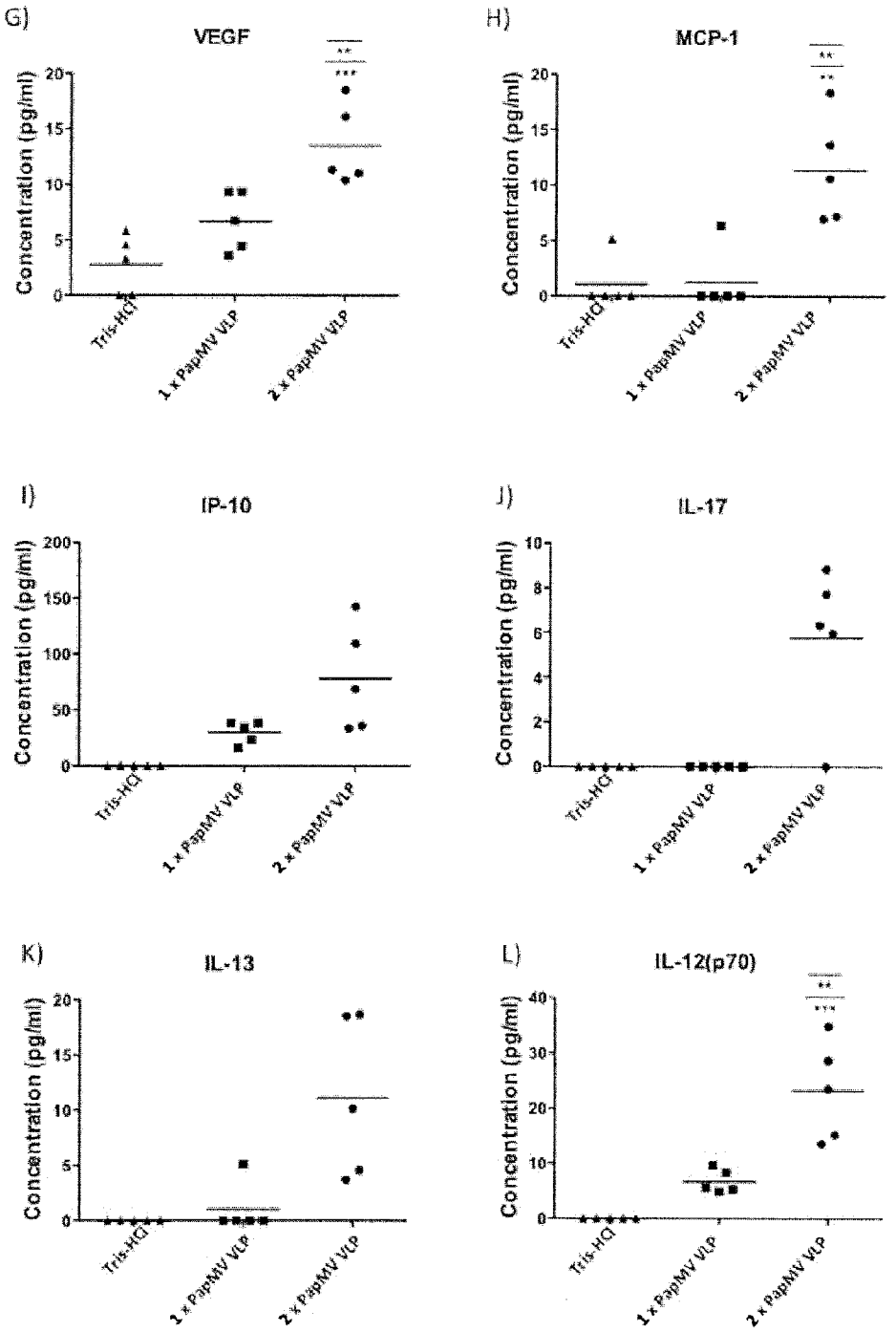

G)
VEGF
Concentration (pg/ml)
Tris-HCl
1 x PapMV VLP
2 x PapMV VLP
H)
MCP-1
I)
IP-10
J)
IL-17
K)
IL-13
L)
IL-12(p70)

FIGURE 7 (con.)

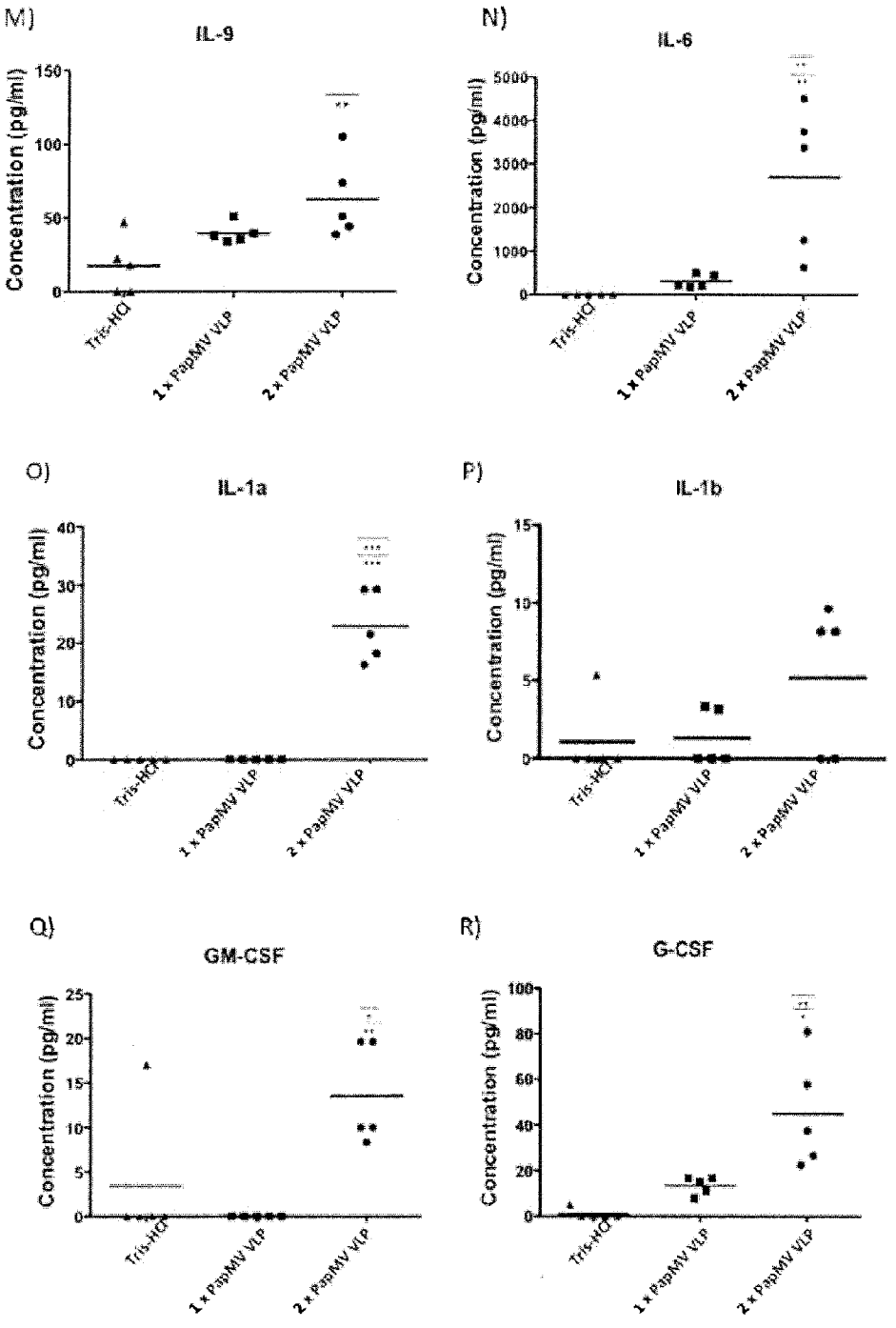
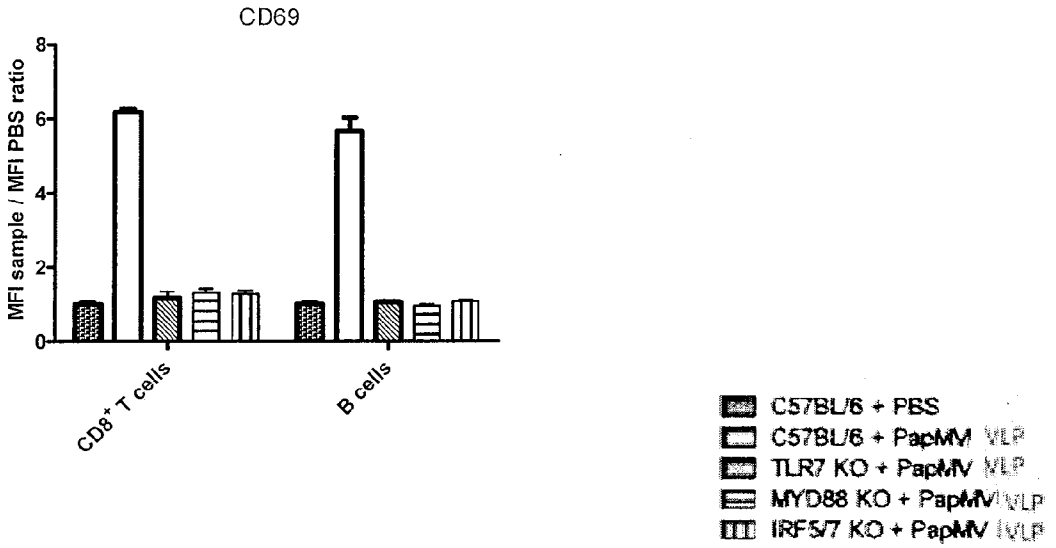

M)
IL-9
Concentration (pg/ml)
150
100
50
0
Tris-HCl
1 x PapMV VLP
2 x PapMV VLP
N)
IL-6
Concentration (pg/ml)
5000
4000
3000
2000
1000
0
Tris-HCl
1 x PapMV VLP
2 x PapMV VLP
O)
IL-1a
Concentration (pg/ml)
40
30
20
10
0
Tris-HCl
1 x PapMV VLP
2 x PapMV VLP
P)
IL-1b
Concentration (pg/ml)
15
10
5
0
Tris-HCl
1 x PapMV VLP
2 x PapMV VLP
Q)
GM-CSF
Concentration (pg/ml)
25
20
15
10
5
0
Tris-HCl
1 x PapMV VLP
2 x PapMV VLP
R)
G-CSF
Concentration (pg/ml)
100
80
60
40
20
0
Tris-HCl
1 x PapMV VLP
2 x PapMV VLP

FIGURE 7 (con.)

A.


CD69
MFI sample / MFI PBS ratio
8
6
4
2
0
CD8+ T cells
B cells
C57BL/6 + PBS
C57BL/6 + PapMV VLP
TLR7 KO + PapMV VLP
MYD88 KO + PapMV VLP
IRF5/7 KO + PapMV VLP

B.

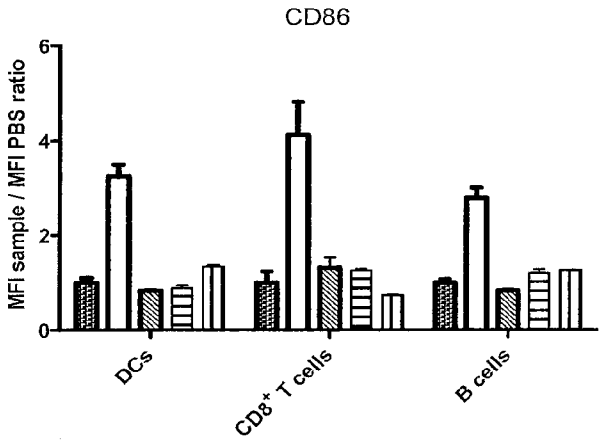

CD86
MFI sample / MFI PBS ratio
6
4
2
0
DCs
CD8+ T cells
B cells

FIGURE 8

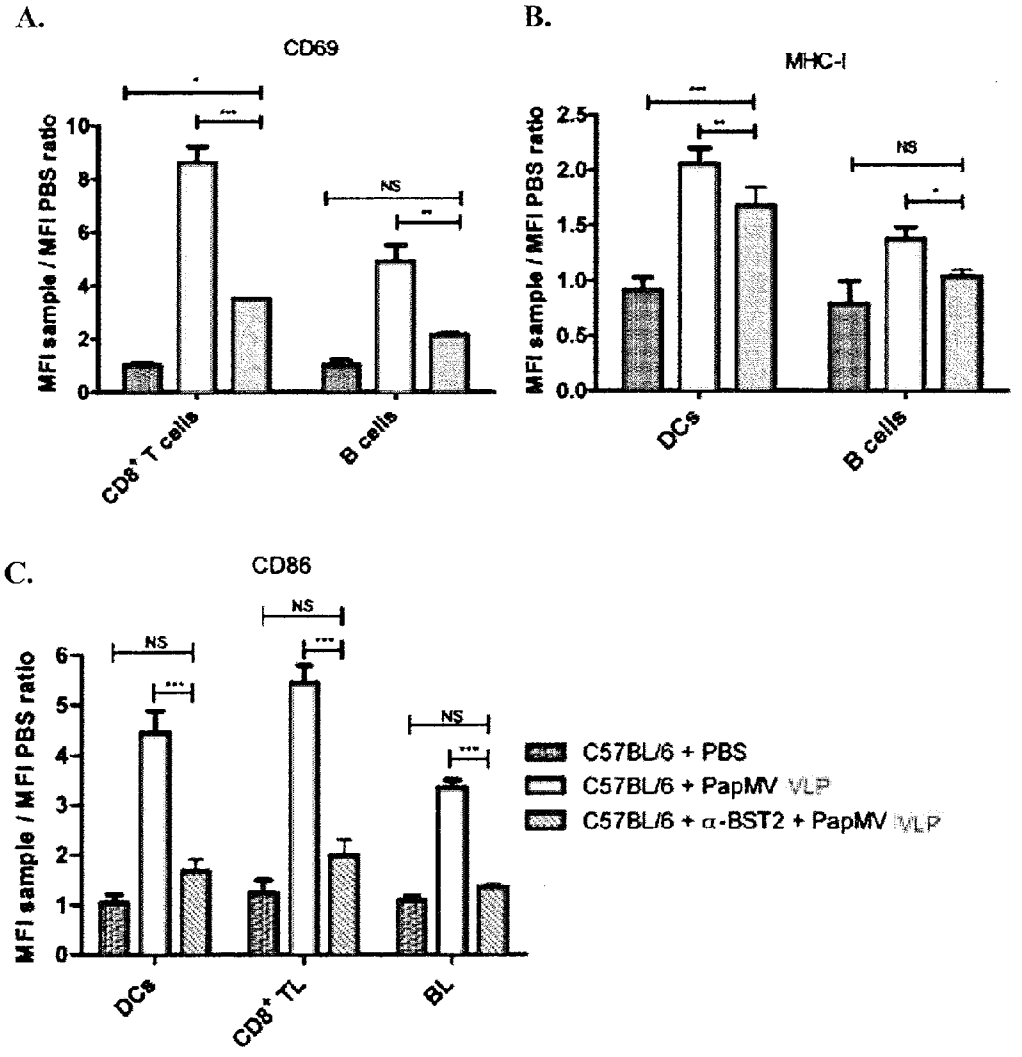

A.
CD69
MFI sample / MFI PBS ratio
CD8+ T cells
B cells
NS
B.
MHC-I
MFI sample / MFI PBS ratio
DCs
B cells
NS
C.
CD86
MFI sample / MFI PBS ratio
DCs
CD8+ TL
BL
NS
C57BL/6 + PBS
C57BL/6 + PapMV VLP
C57BL/6 + α-BST2 + PapMV VLP

FIGURE 9

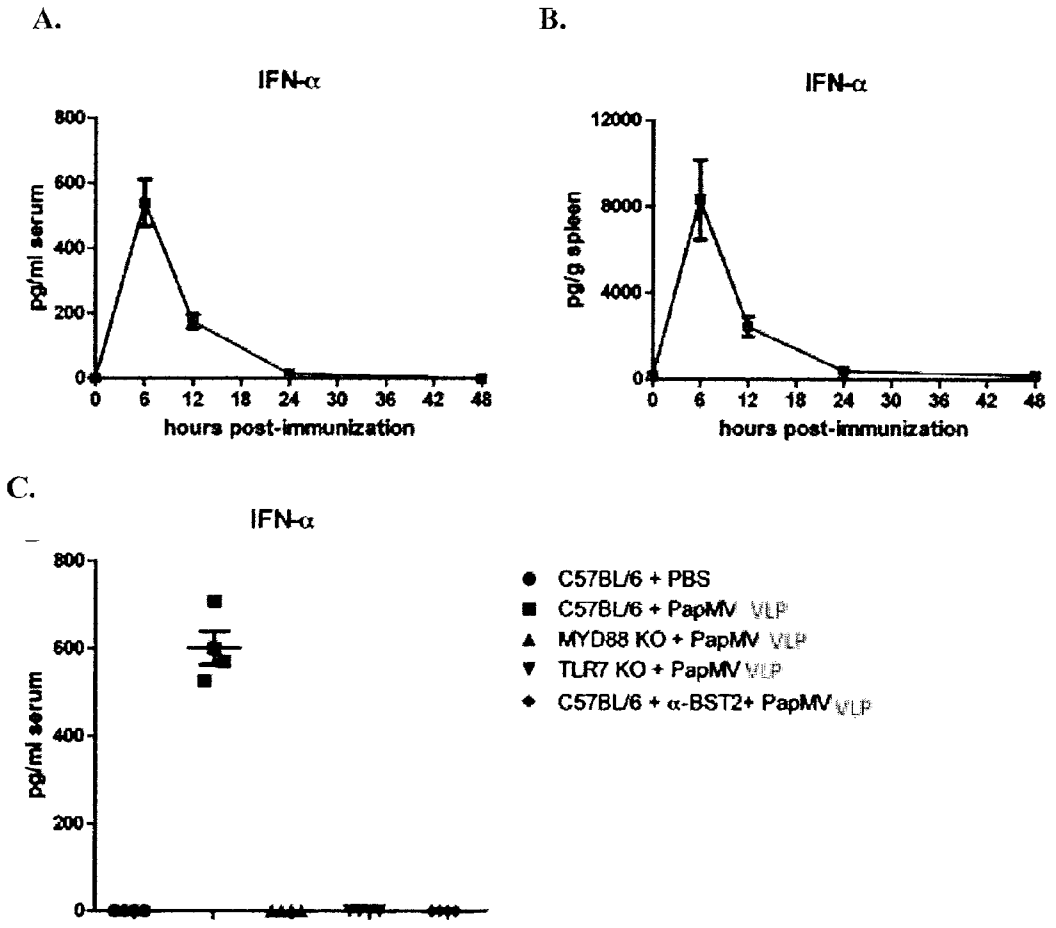

A.
IFN-α
pg/ml serum
800
600
400
200
0
0 6 12 18 24 30 36 42 48
hours post-immunization
B.
IFN-α
pg/g spleen
12000
8000
4000
0
0 6 12 18 24 30 36 42 48
hours post-immunization
C.
IFN-α
pg/ml serum
800
600
400
200
0
C57BL/6 + PBS
C57BL/6 + PapMV VLP
MYD88 KO + PapMV VLP
TLR7 KO + PapMV VLP
C57BL/6 + α-BST2+ PapMV VLP

FIGURE 10

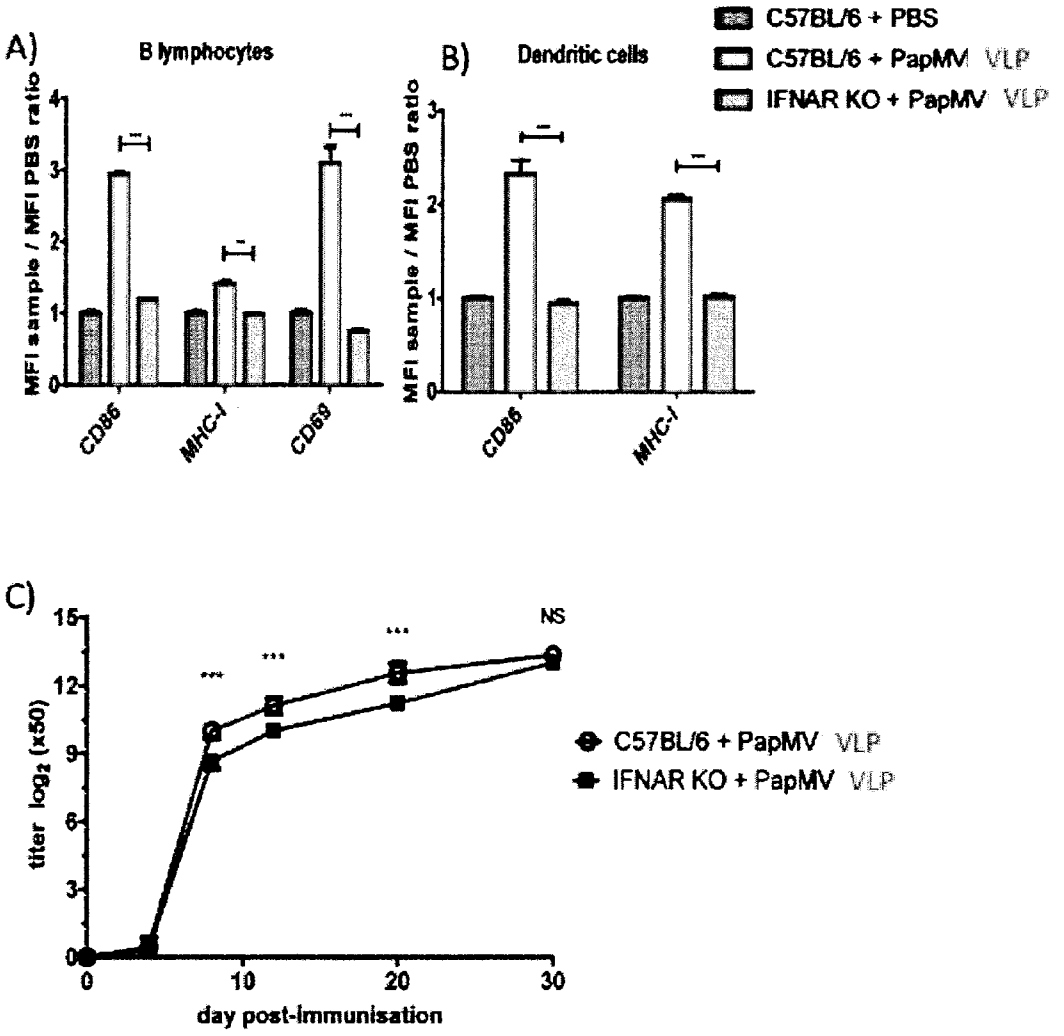

A)
B lymphocytes
B)
Dendritic cells
C57BL/6 + PBS
C57BL/6 + PapMV VLP
IFNAR KO + PapMV VLP
MFI sample / MFI PBS ratio
CD86
MHC-I
CD69
C)
titer log2 (x50)
day post-immunisation
***
NS

FIGURE 11

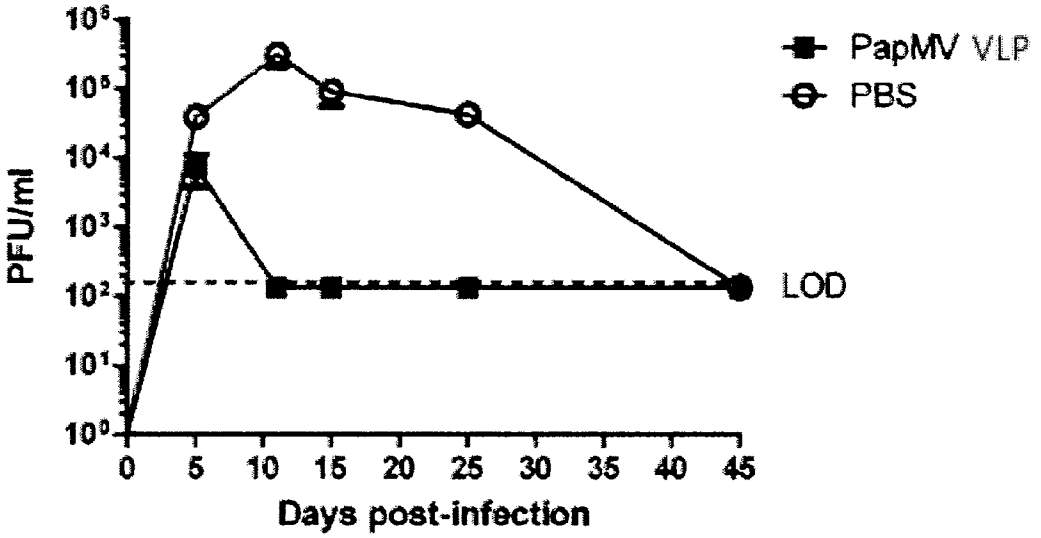

PapMV VLP
PBS
LOD
PFU/ml
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0 5 10 15 20 25 30 35 40 45
Days post-infection

FIGURE 12

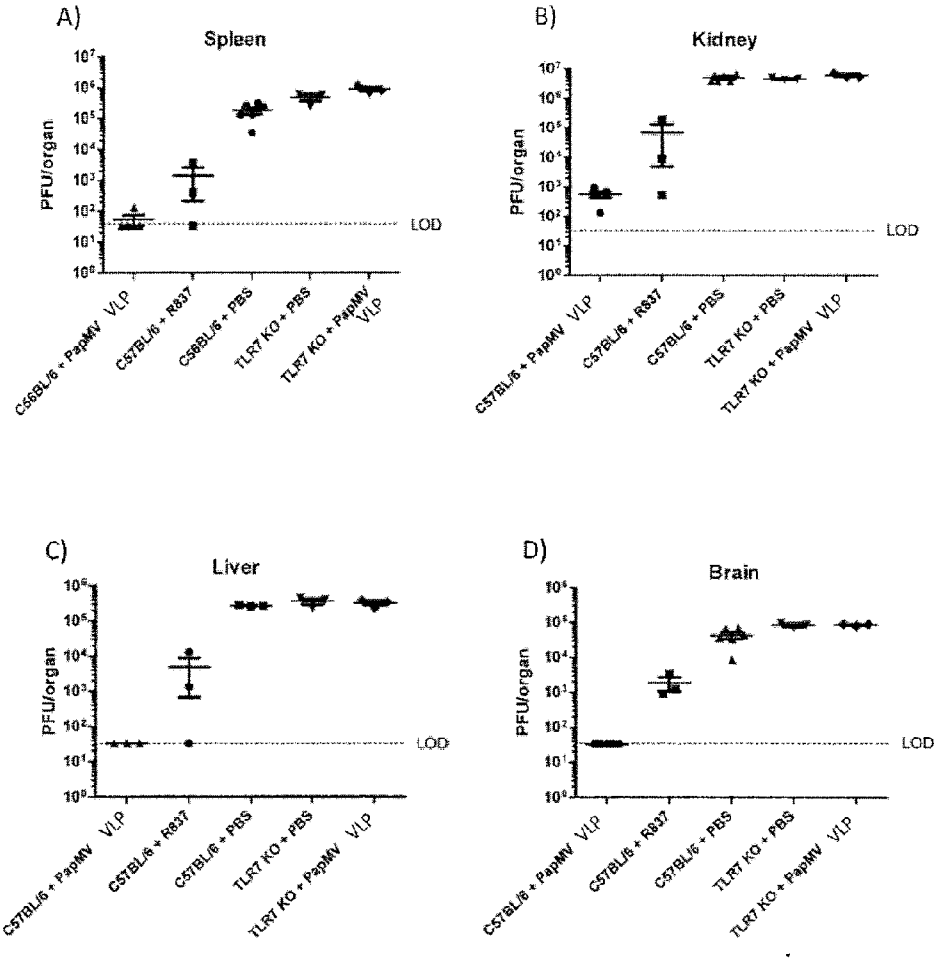

A)
Spleen
PFU/organ
LOD
C56BL/6 + PapMV VLP
C57BL/6 + R837
C56BL/6 + PBS
TLR7 KO + PBS
TLR7 KO + PapMV VLP
B)
Kidney
PFU/organ
LOD
C57BL/6 + PapMV VLP
C57BL/6 + R837
C57BL/6 + PBS
TLR7 KO + PBS
TLR7 KO + PapMV VLP
C)
Liver
PFU/organ
LOD
C57BL/6 + PapMV VLP
C57BL/6 + R837
C57BL/6 + PBS
TLR7 KO + PBS
TLR7 KO + PapMV VLP
D)
Brain
PFU/organ
LOD
C57BL/6 + PapMV VLP
C57BL/6 + R837
C57BL/6 + PBS
TLR7 KO + PBS
TLR7 KO + PapMV VLP

FIGURE 13

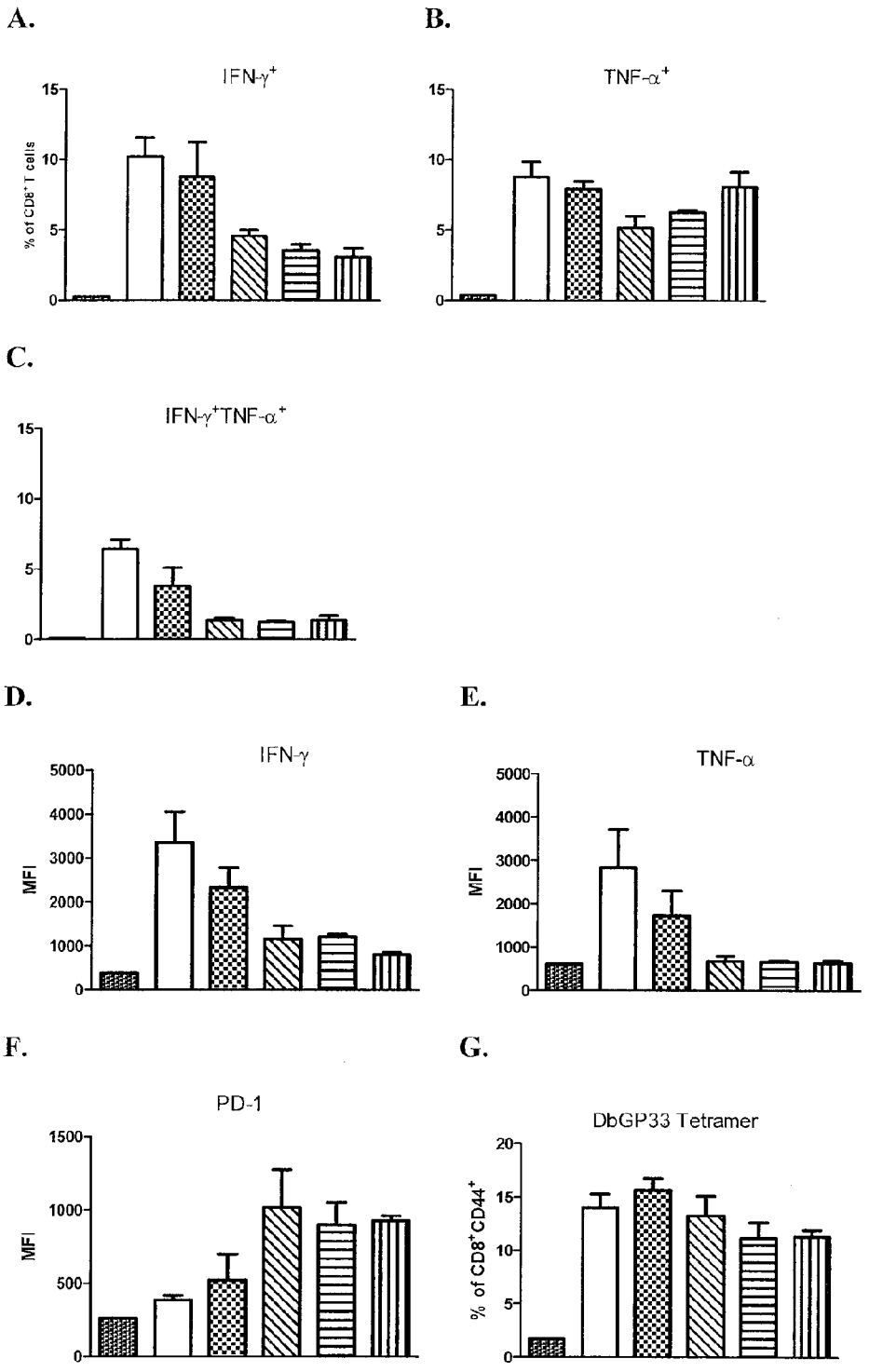

A.
IFN-γ+
% of CD8+ T cells
15
10
5
0
B.
TNF-α+
15
10
5
0
C.
IFN-γ+TNF-α+
15
10
5
0
D.
IFN-γ
MFI
5000
4000
3000
2000
1000
0
E.
TNF-α
MFI
5000
4000
3000
2000
1000
0
F.
PD-1
MFI
1500
1000
500
0
G.
DbGP33 Tetramer
% of CD8+CD44+
20
15
10
5
0

FIGURE 14

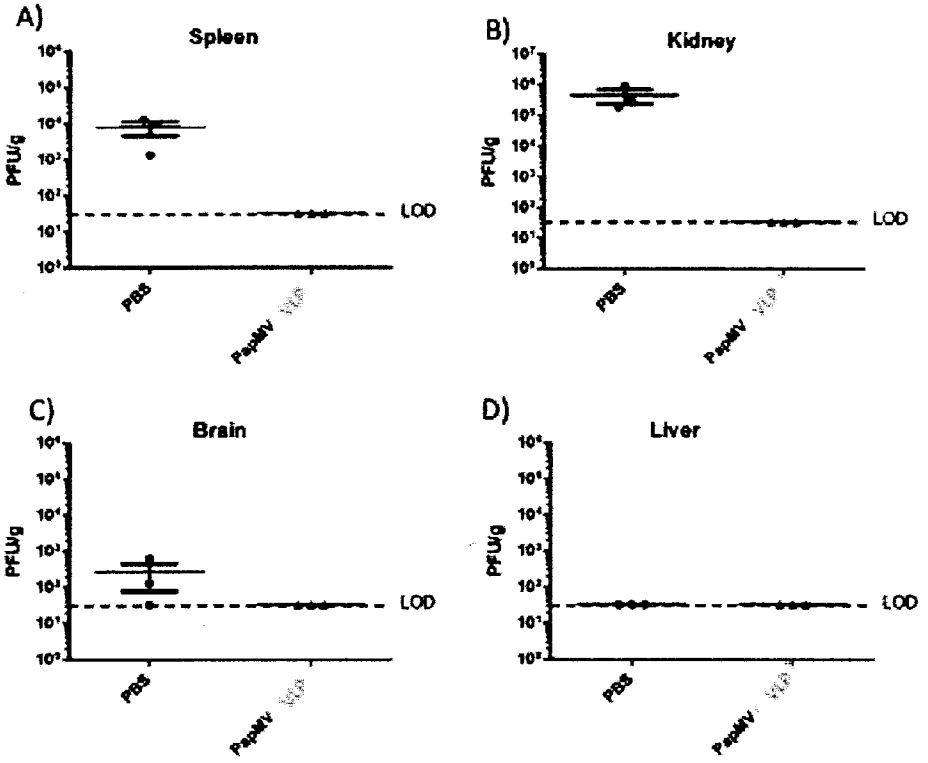

A)
Spleen
PFU/g
LOD
PBS
B)
Kidney
PFU/g
LOD
PBS
C)
Brain
PFU/g
LOD
PBS
D)
Liver
PFU/g
LOD
PBS

FIGURE 15

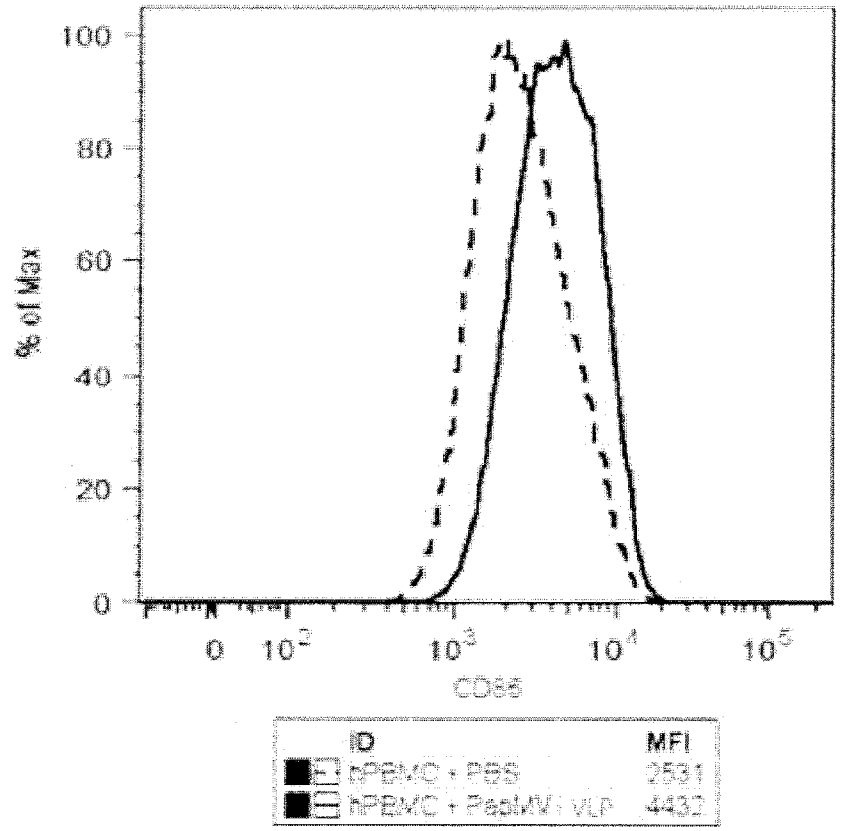

100
80
60
40
20
0
% of Max
0
10^2
10^3
10^4
10^5
CD86
ID
MFI
hPBMC + PBS
2531
hPBMC + PadMV-VLP
4432

FIGURE 16

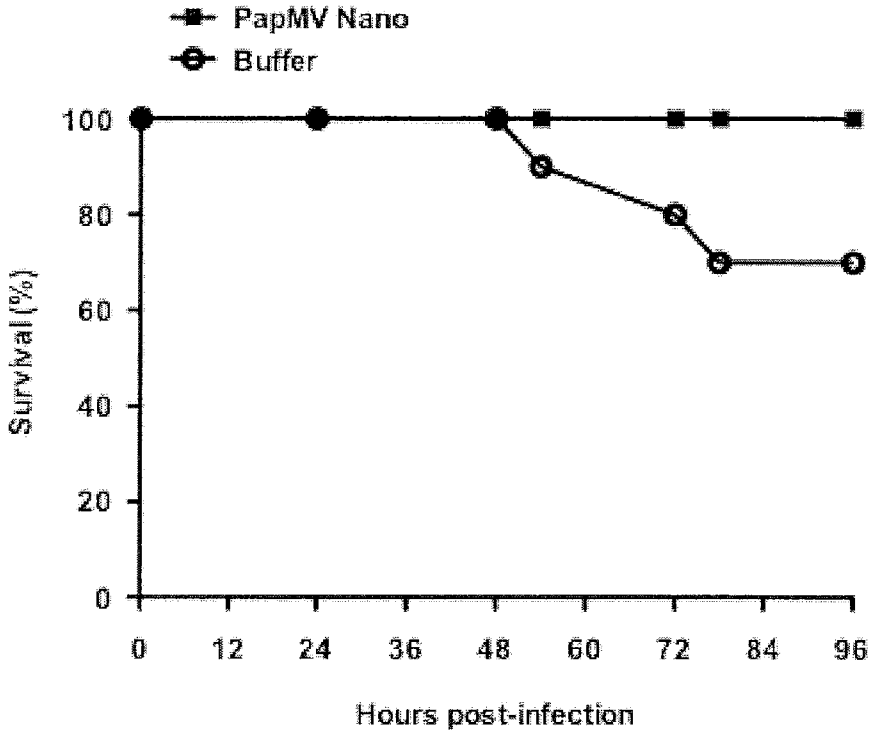

PapMV Nano
Buffer
100
80
60
40
20
0
Survival (%)
0
12
24
36
48
60
72
84
96
Hours post-infection

FIGURE 17

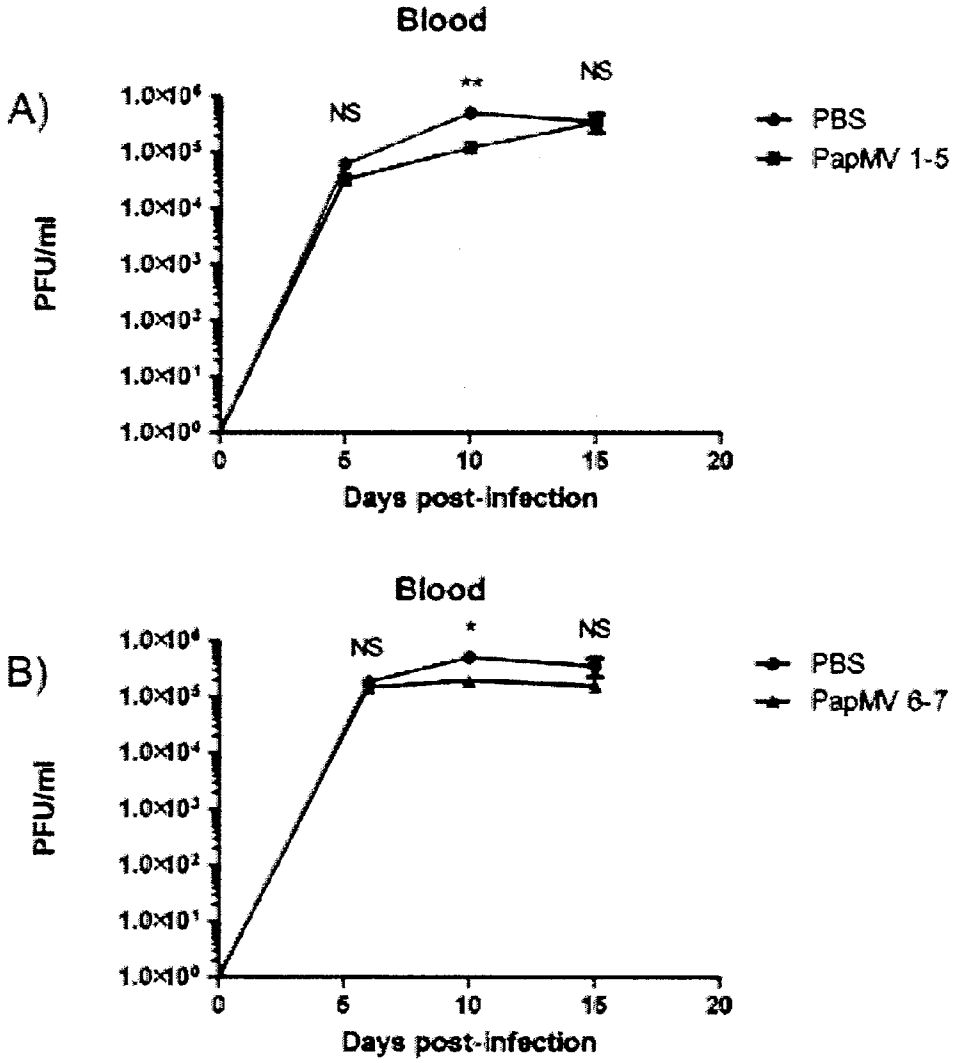

A)
Blood
PFU/ml
1.0×10⁶
1.0×10⁵
1.0×10⁴
1.0×10³
1.0×10²
1.0×10¹
1.0×10⁰
NS
**
NS
0
5
10
15
20
Days post-infection
PBS
PapMV 1-5
B)
Blood
PFU/ml
1.0×10⁶
1.0×10⁵
1.0×10⁴
1.0×10³
1.0×10²
1.0×10¹
1.0×10⁰
NS
*
NS
0
5
10
15
20
Days post-infection
PBS
PapMV 6-7

FIGURE 18

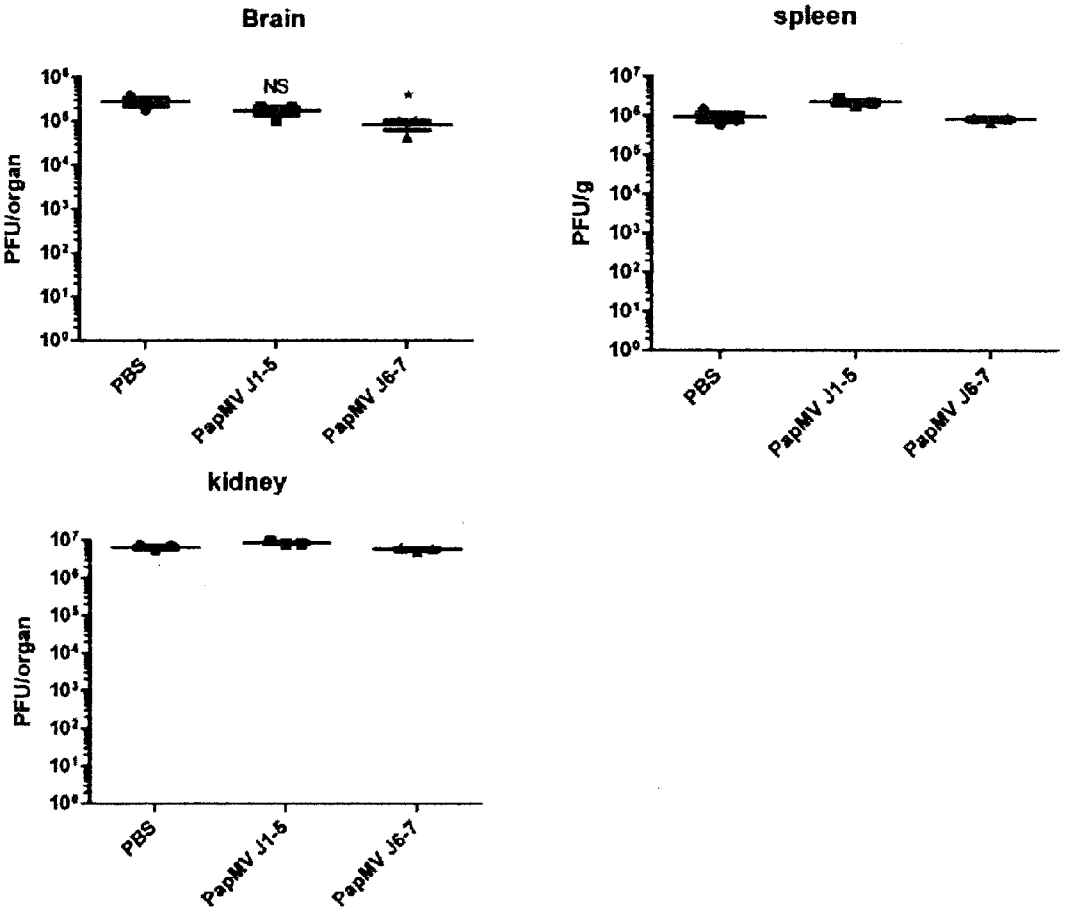

Brain
PFU/organ
NS
*
PBS
PapMV J1-5
PapMV J6-7
spleen
PFU/g
PBS
PapMV J1-5
PapMV J6-7
kidney
PFU/organ
PBS
PapMV J1-5
PapMV J6-7

FIGURE 19

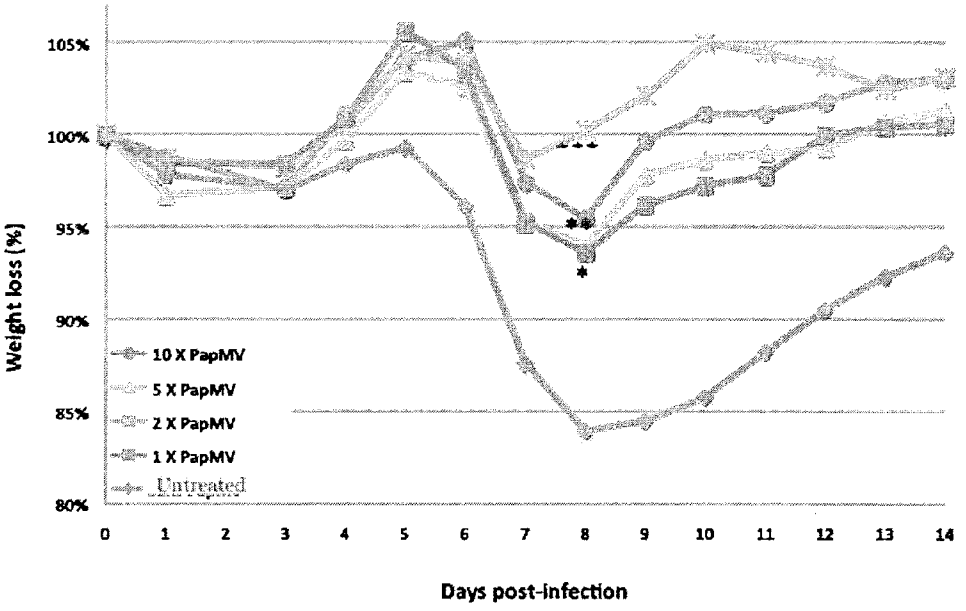

105%
100%
95%
90%
85%
80%
Weight loss (%)
10 X PapMV
5 X PapMV
2 X PapMV
1 X PapMV
Untreated
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Days post-infection

FIGURE 20

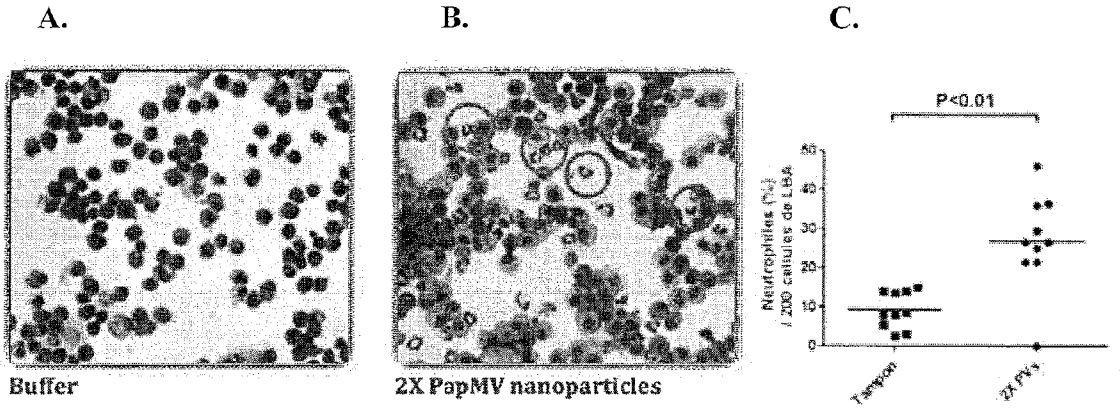

A.
B.
C.
Buffer
2X PapMV nanoparticles
P<0.01
Neutrophiles (%)
/ 200 cellules de LBA
0
15
30
45
Tampon
2X PN's

FIGURE 21

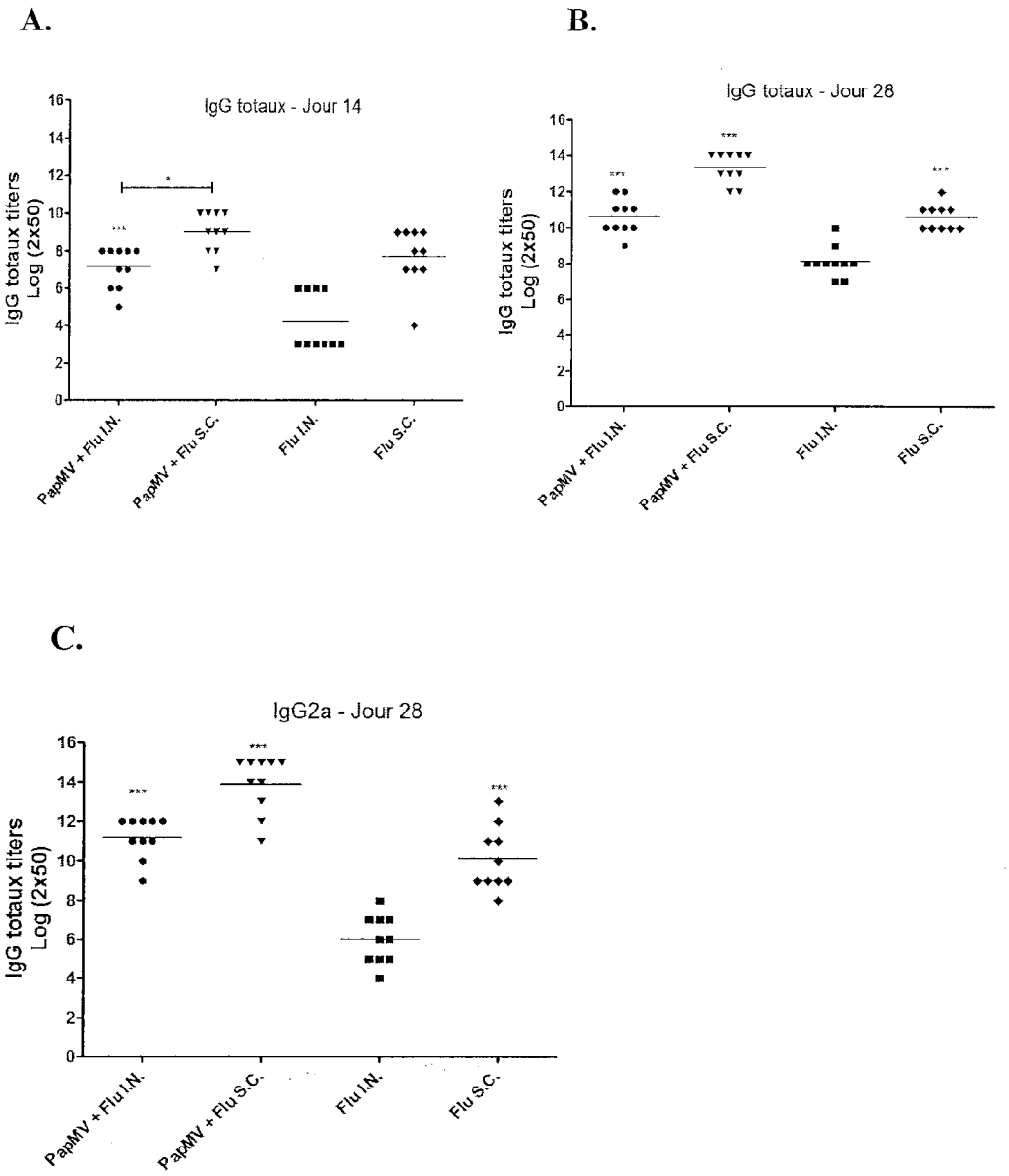

A.
IgG totaux - Jour 14
IgG totaux titers Log (2x50)
PapMV + Flu I.N.
PapMV + Flu S.C.
Flu I.N.
Flu S.C.
B.
IgG totaux - Jour 28
IgG totaux titers Log (2x50)
PapMV + Flu I.N.
PapMV + Flu S.C.
Flu I.N.
Flu S.C.
C.
IgG2a - Jour 28
IgG totaux titers Log (2x50)
PapMV + Flu I.N.
PapMV + Flu S.C.
Flu I.N.
Flu S.C.

FIGURE 22

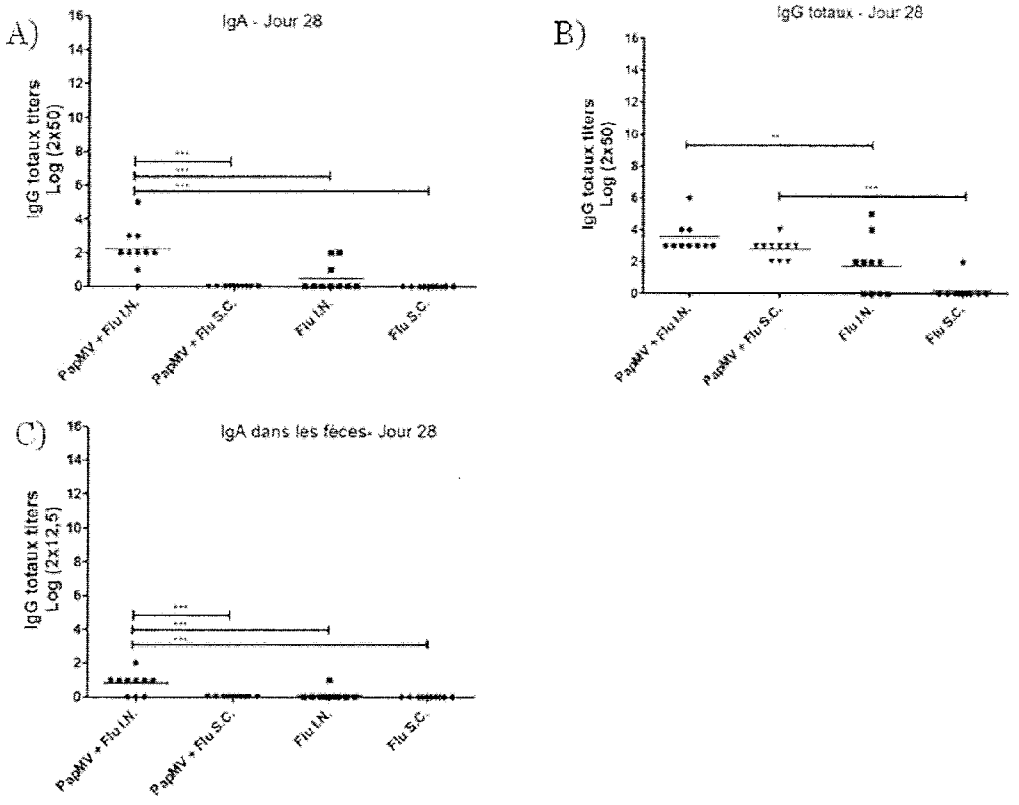

A)
IgA - Jour 28
IgG totaux titers
Log (2x50)
PapMV + Flu I.N.
PapMV + Flu S.C.
Flu I.N.
Flu S.C.
B)
IgG totaux - Jour 28
IgG totaux titers
Log (2x50)
PapMV + Flu I.N.
PapMV + Flu S.C.
Flu I.N.
Flu S.C.
C)
IgA dans les fèces- Jour 28
IgG totaux titers
Log (2x12,5)
PapMV + Flu I.N.
PapMV + Flu S.C.
Flu I.N.
Flu S.C.

FIGURE 23

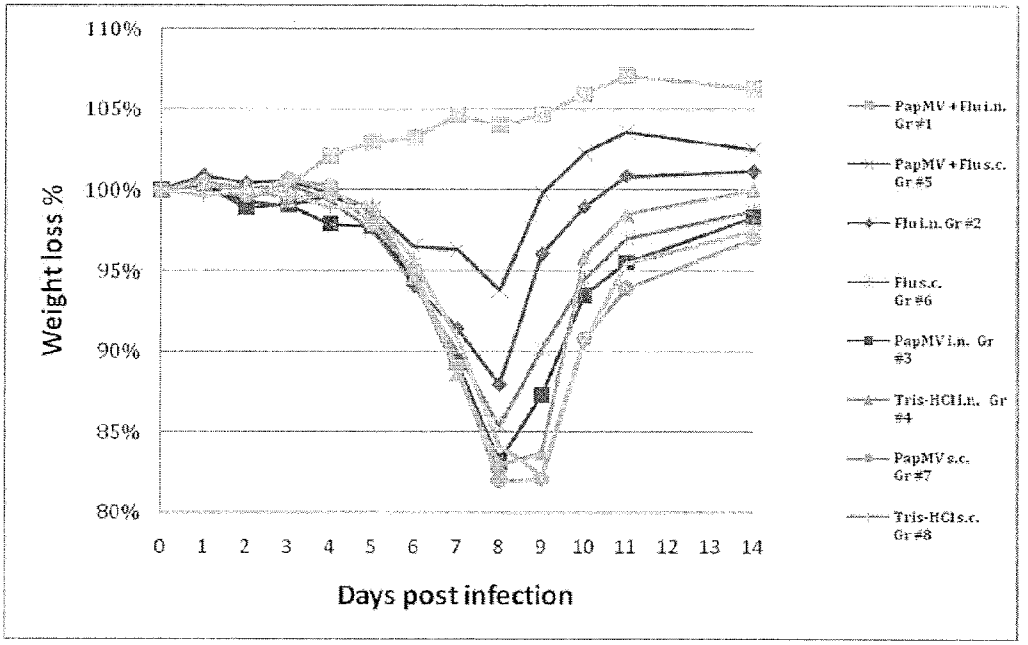
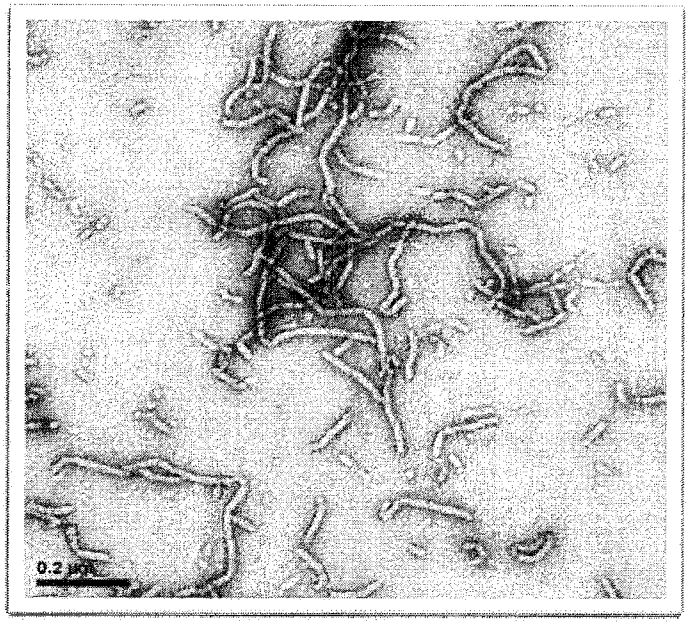

110%
105%
100%
95%
90%
85%
80%
Weight loss %
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days post infection
PapMV + Flu i.n. Gr#1
PapMV + Flu s.c. Gr#5
Flu i.n. Gr#2
Flu s.c. Gr#6
PapMV i.n. Gr #3
Tris-HCl i.n. Gr #4
PapMV s.c. Gr#7
Tris-HCl s.c. Gr#8

FIGURE 24

A.



B.

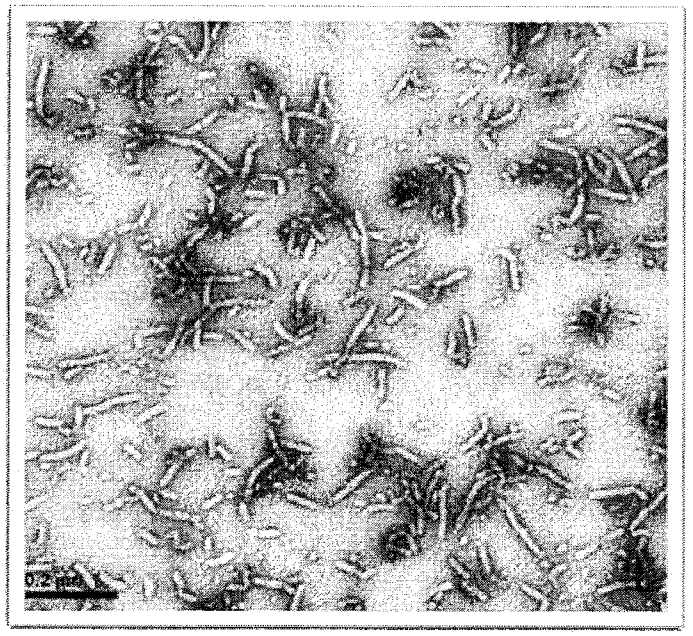


FIGURE 25

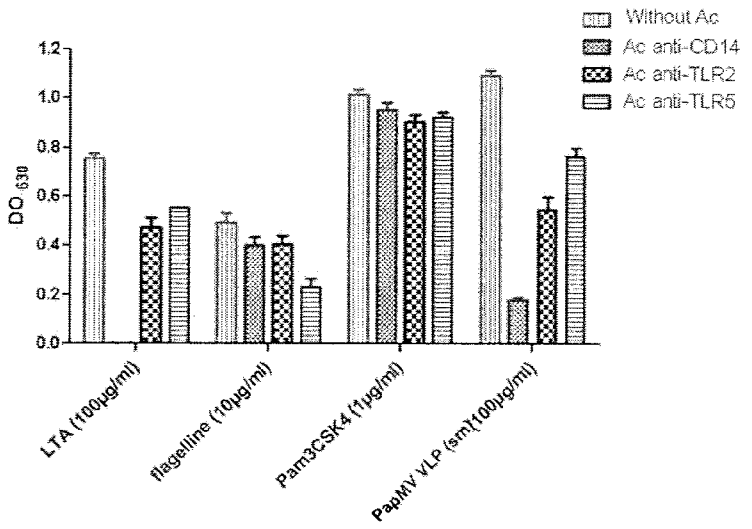

Without Ac
Ac anti-CD14
Ac anti-TLR2
Ac anti-TLR5
DO 630
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LTA (100µg/ml)
flagelline (10µg/ml)
Pam3CSK4 (1µg/ml)
PapMV VLP (sm)(100µg/ml)

FIGURE 26

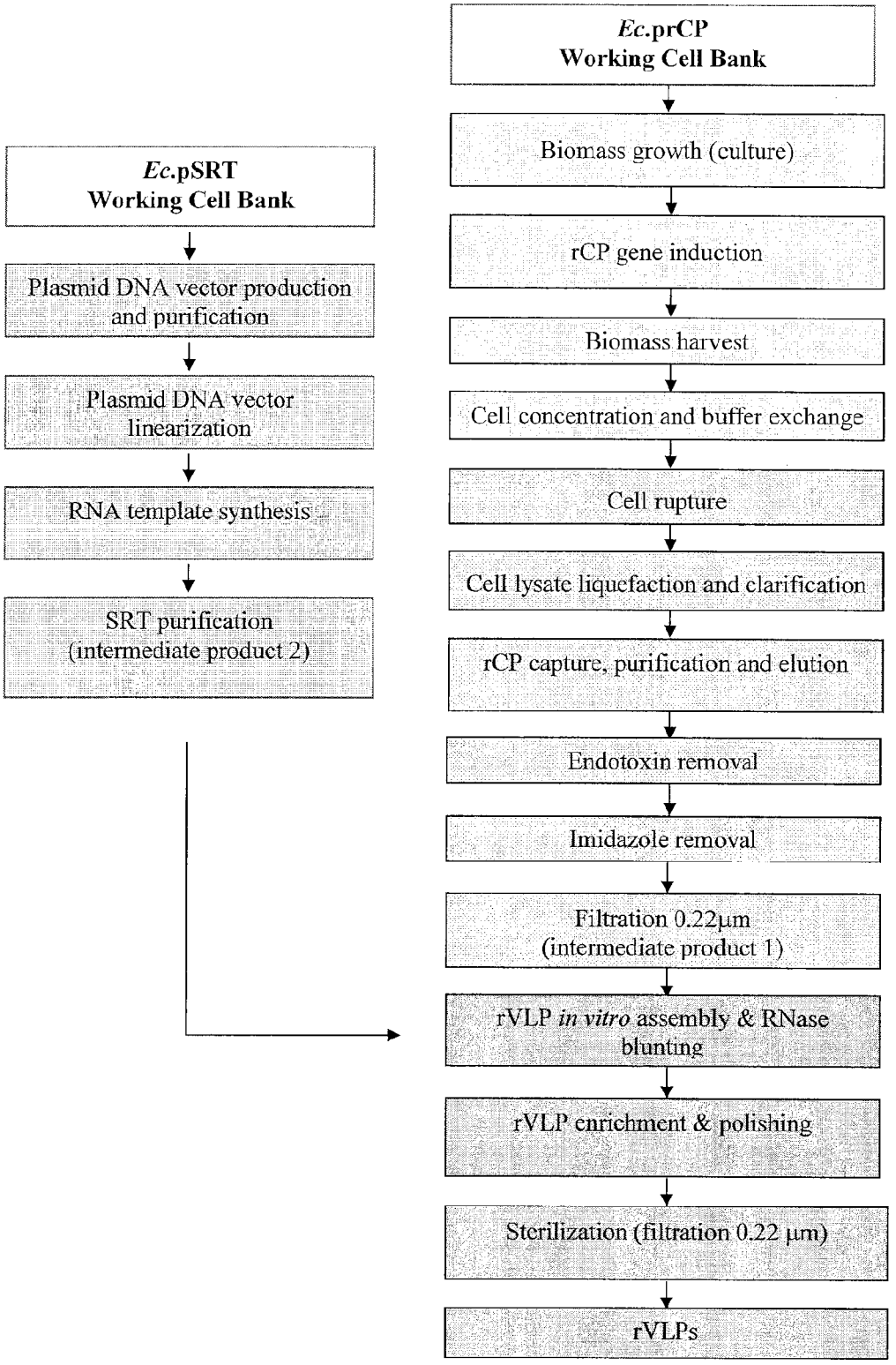
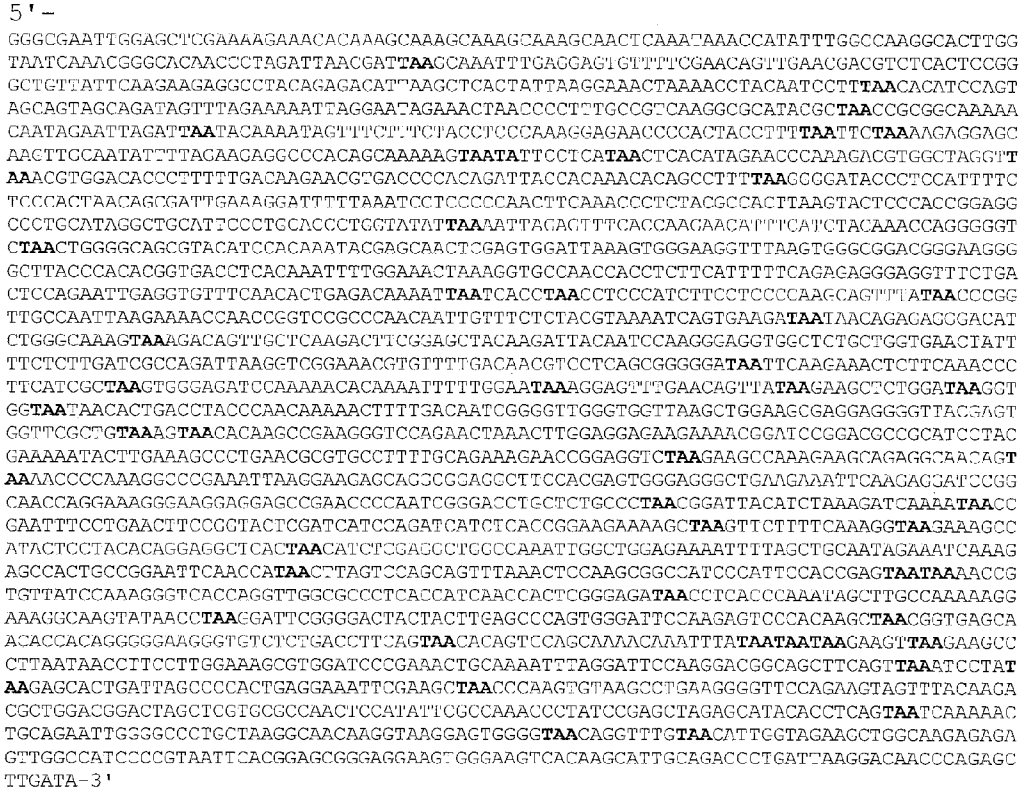

*Ec.*pSRT
Working Cell Bank
Plasmid DNA vector production and purification
Plasmid DNA vector linearization
RNA template synthesis
SRT purification (intermediate product 2)
*Ec.*prCP
Working Cell Bank
Biomass growth (culture)
rCP gene induction
Biomass harvest
Cell concentration and buffer exchange
Cell rupture
Cell lysate liquefaction and clarification
rCP capture, purification and elution
Endotoxin removal
Imidazole removal
Filtration 0.22µm (intermediate product 1)
rVLP *in vitro* assembly & RNase blunting
rVLP enrichment & polishing
Sterilization (filtration 0.22 µm)
rVLPs

FIGURE 27

T7-TRANSCRIPTION START-
GGGCGAATTGGAGCTCGAAAAGAAACACAAAGCAAAGCAAAGCAAAGCAACTCAAATAAACCATATTTGGCCAAGCCACTTGG
TAATCAAACGGGCACAACCCTAGATTAACGAT**TAA**GCAAATTTGAGGAGTGTTTTCGAACAGTTGAACGACGTCTCACTCCGG
GCTGTTATTCAAGAAGAGGCCTACAGAGACATTAAGCTCACTATTAAGGAAACTAAAACCTACAATCCTT**TAA**CACATCCAGT
AGCAGTAGCAGATAGTTTAGAAAAATTAGGAATAGAAACTAACCCCTTTGCCGTCAAGGCGCATACGC**TAA**CCGCGGCAAAAA
CAATAGAATTAGAT**TAA**TACAAAATAGTTTCTTTCTACCTCCCAAAGGAGAACCCCACTACCTTT**TAA**TTC**TAA**AAGAGGAGC
AAGTTGCAATATTTTAGAAGAGGCCCACAGCAAAAAG**TAATA**TTCCTCA**TAA**CTCACATAGAACCCAAAGACGTGGCTAGGT**T**
AAACGTGGACACCCTTTTTGACAAGAACGTGACCCCACAGATTACCACAAACACAGCCTTT**TAA**GGGGATACCCTCCATTTTC
TCCCACTAACAGCGATTGAAAGGATTTTTAAATCCTCCCCCAACTTCAAACCCTCTACGCCACT**TAA**GTACTCCCACCGGAGG
CCCTGCATAGGCTGCATTCCCTGCACCCTGGTATAT**TAA**AATTAGAGTTTCACCAAGAACATTTCATCTACAAACCAGGGGGT
C**TAA**CTGGGGCAGCGTACATCCACAAATACGAGCAACTCGAGTGGATTAAAGTGGGAAGGTTTAAGTGGGCGGACGGGAAGGG
GCTTACCCACACGGTGACCTCACAAATTTTGGAAACTAAAGGTGCCAACCACCTCTTCATTTTTCAGAGAGGGAGGTTTCTGA
CTCCAGAATTGAGGTGTTTCAACACTGAGACAAAAT**TAA**TCACC**TAA**CCTCCCATCTTCCTCCCCAAGCAGTTTA**TAA**CCCGG
TTGCCAATTAAGAAAACCAACCGGTCCGCCCAACAATTGTTTCTCTACGTAAAATCAGTGAAGA**TAA**TAACAGAGAGGGACAT
CTGGGCAAAG**TAA**AGACAGTTGCTCAAGACTTCGGAGCTACAAGATTACAATCCAAGGGAGGTGGCTCTGCTGGTGAACTATT
TTCTCTTGATCGCCAGATTAAGGTCGGAAACGTGTTTTGACAACGTCCTCAGCGGGGGA**TAA**TTCAAGAAACTCTTCAAACCC
TTCATCGC**TAA**GTGGGAGATCCAAAAACACAAAATTTTTGGAA**TAA**AGGAGTTTGAACAGTTA**TAA**GAAGCTCTGGA**TAA**GGT
GG**TAA**TAACACTGACCTACCCAACAAAAACTTTTGACAATCGGGGTTGGGTGGTTAAGCTGGAAGCGAGGAGGGGTTACGAGT
GGTTCGCTG**TAA**AG**TAA**CACAAGCCGAAGGGTCCAGAACTAAACTTGGAGGAGAAGAAAACGGATCCGGACGCCGCATCCTAC
GAAAAATACTTGAAAGCCCTGAACGCGT-3'

FIGURE 28


5'-
GGGCGAATTGGAGCTCGAAAAGAAACACAAAGCAAAGCAAAGCAAAGCAACTCAAATAAACCATATTTGGCCAAGGCACTTGG
TAATCAAACGGGCACAACCCTAGATTAACGATTAAGCAAATTTGAGGAGTGTTTTCGAACAGTTGAACGACGTCTCACTCCGG
GCTGTTATTCAAGAAGAGGCCTACAGAGACATTAAGCTCACTATTAAGGAAACTAAAACCTACAATCCTTTAACACATCCAGT
AGCAGTAGCAGATAGTTTAGAAAAATTAGGAATAGAAACTAACCCCTTTGCCGTCAAGGCGCATACGCTAACCGCGGCAAAAA
CAATAGAATTAGATTAATACAAAATAGTTTCTTTCTACCTCCCAAAGGAGAACCCCACTACCTTTTAATTCTAAAAGAGGAGC
AAGTTGCAATATTTTAGAAGAGGCCCACAGCAAAAAGTAATATTCCTCATAACTCACATAGAACCCAAAGACGTGGCTAGGTT
AAACGTGGACACCCTTTTTGACAAGAACGTGACCCCACAGATTACCACAAACACAGCCTTTTAACGGGATACCCTCCATTTTC
TCCCACTAACAGCGATTGAAAGGATTTTTAAATCCTCCCCCAACTTCAAACCCTCTACGCCACTTAAGTACTCCCACCGGAGG
CCCTGCATAGGCTGCATTCCCTGCACCCTGGTATATTAAAATTACAGTTTCACCAAGAACATTTCATCTACAAACCAGGGGGT
CTAACTGGGGCAGCGTACATCCACAAATACGAGCAACTCGAGTGGATTAAAGTGGGAAGGTTTAAGTGGGCGGACGGGAAGGG
GCTTACCCACACGGTGACCTCACAAATTTTGGAAACTAAAGGTGCCAACCACCTCTTCATTTTTCAGACAGGGAGGTTTCTGA
CTCCAGAATTGAGGTGTTTCAACACTGAGACAAAATTAATCACCTAACCTCCCATCTTCCTCCCCAAGCAGTTTATAACCCGG
TTGCCAATTAAGAAAACCAACCGGTCCGCCCAACAATTGTTTCTCTACGTAAAATCAGTGAAGATAATAACAGAGAGGGACAT
CTGGGCAAAGTAAAGACAGTTGCTCAAGACTTCGGAGCTACAAGATTACAATCCAAGGGAGGTGGCTCTGCTGGTGAACTATT
TTCTCTTGATCGCCAGATTAAGGTCGGAAACGTGTTTTGACAACGTCCTCAGCGGGGGATAATTCAAGAAACTCTTCAAACCC
TTCATCGCTAAGTGGGAGATCCAAAAACACAAAATTTTTGGAATAAAGGAGTTTGAACAGTTATAAGAAGCTCTGGATAAGGT
GGTAATAACACTGACCTACCCAACAAAAACTTTTGACAATCGGGGTTGGGTGGTTAAGCTGGAAGCGAGGAGGGGTTACGAGT
GGTTCGCTGTAAAGTAACACAAGCCGAAGGGTCCAGAACTAAACTTGGAGGAGAAGAAAACGGATCCGGACGCCGCATCCTAC
GAAAAATACTTGAAAGCCCTGAACGCGTGCCTTTTGCAGAAAGAACCGGAGGTCTAAGAAGCCAAAGAAGCAGAGGCAACAGT
AAAACCCCAAAGGCCCGAAATTAAGGAAGAGCAGCGCGAGCCTTCCACGAGTGGGAGGGCTGAAGAAATTCAAGAGGATCCGG
CAACCAGGAAAGGGAAGGAGGAGCCGAACCCCAATCGGGACCTGCTCTGCCCTAACGGATTACATCTAAAGATCAAAATAACC
GAATTTCCTGAACTTCCGGTACTCGATCATCCAGATCATCTCACCGGAAGAAAAGCTAAGTTCTTTTCAAAGGTAAGAAAGCC
ATACTCCTACACAGGAGGCTCACTAACATCTCGAGCCTGGCCAAATTGGCTGGAGAAAATTTTAGCTGCAATAGAAATCAAAG
AGCCACTGCCGGAATTCAACCATAACTTAGTCCAGCAGTTTAAACTCCAAGCGGCCATCCCATTCCACCGAGTAATAAAACCG
TGTTATCCAAAGGGTCACCAGGTTGGCGCCCTCACCATCAACCACTCGGGAGATAACCTCACCCAAATAGCTTGCCAAAAAGG
AAAGGCAAGTATAACCTAAGGATTCGGGGACTACTACTTGAGCCCAGTGGGATTCCAAGAGTCCCACAAGCTAACGGTGAGCA
ACACCACAGGGGGAAGGGTGTCTCTGACCTTCAGTAACACAGTCCAGCAAAACAAATTTATAATAATAAGAAGTTAAGAAGCC
CTTAATAACCTTCCTTGGAAAGCGTGGATCCCGAAACTGCAAAATTTAGGATTCCAAGGACGGCAGCTTCAGTTAAATCCTAT
AAGAGCACTGATTAGCCCCACTGAGGAAATTCGAAGCTAACCCAAGTGTAAGCCTGAAGGGGTTCCAGAAGTAGTTTACAAGA
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TGCAGAATTGGGGCCCTGCTAAGGCAACAAGGTAAGGAGTGGGGTAACAGGTTTGTAACATTCGTAGAAGCTGGCAAGAGAGA
GTTGGCCATCCCCGTAATTCACGGAGCGGGAGGAAGTGGGAAGTCACAAGCATTGCAGACCCTGATTAAGGACAACCCAGAGC
TTGATA-3'

FIGURE 29

PAPAYA MOSAIC VIRUS COMPOSITIONS AND USES THEREOF FOR STIMULATION OF THE INNATE IMMUNE RESPONSE

FIELD OF THE INVENTION

[0001] The present invention relates to the field of immunomodulation and, in particular, to the use of compositions comprising papaya mosaic virus (PapMV) or PapMV virus-like particles to stimulate the innate immune response.

BACKGROUND OF THE INVENTION

[0002] The ability of papaya mosaic virus (PapMV) and PapMV virus-like particles (VLPs) to enhance the immunogenicity of antigens has been described in the following patent and patent applications.

[0003] U.S. Pat. No. 7,641,896, Canadian Patent Application No. 2,434,000, and International Patent Application No. PCT/CA03/00985 (WO 2004/004761) describe the use of PapMV or VLPs derived from PapMV coat protein for potentiating an immune response to an antigen in an animal. The antigen(s) may be attached to the PapMV or VLP or they may be administered in combination with the PapMV or VLP.

[0004] International Patent Application No. PCT/CA2007/002069 (WO 2008/058396) describes influenza vaccines based on PapMV and PapMV VLPs. The vaccines comprise PapMV or a PapMV VLP and one or more influenza antigens, which may be attached to the PapMV or VLP or may be administered in combination with the PapMV or VLP.

[0005] International Patent Application No. PCT/CA2007/001904 (WO 2008/058369) describes immunogenic affinity-conjugated antigen systems based on PapMV. This application describes fusions of PapMV coat protein with a plurality of affinity peptides capable of binding an antigen of interest.

[0006] International Patent Application No. PCT/CA2008/000154 (WO 2008/089569) describes vaccines against *S. typhi* and other enterobacterial pathogens based on PapMV. The vaccines comprise PapMV or a PapMV VLP and one or more enterobacterial antigens, which may be attached to the PapMV or VLP or may be administered in combination with the PapMV or VLP.

[0007] International Patent Application No. PCT/CA2009/00636 (WO 2010/012069) describes multivalent vaccines that comprise a PapMV component and one or more antigens, and their use to provide protection against a plurality of strains of a pathogen, or against more than one pathogen. The vaccines can optionally comprise a *Salmonella* spp. porin component.

[0008] Other publications have described the ability of PapMV VLPs to elicit humoral and cellular immune responses to antigens (Denis et al., 2007, *Virology,* 363: 59-68; Denis et al., 2008, *Vaccine* 26: 3395-403; Leclerc et al., 2007, *J Virol,* 81: 1319-26; Lacasse et al., 2008, *J. Virol,* 82: 785-94). PapMV virus particles isolated from plants have been shown to activate the innate immune response and to be recognized by the immune system as a pathogen associated molecular pattern (PAMP), leading to the proposal that the immunogenicity and intrinsic adjuvant properties of PapMV translate into the specific long-lasting antibody response observed when PapMV is co-administered with antigens (Acosta-Ramirez et al., 2008, *Immunology,* 124: 186-97).

[0009] Innate immunity is the first line of antibody-independent defense against infections and, in many instances, can eliminate infectious agents. The components of innate immunity recognize structures that are characteristic of microbial pathogens and are not present on mammalian cells. The principle effector cells of innate immunity are neutrophils, mononuclear phagocytes, and natural killer (NK) cells. Neutrophils and macrophages express surface receptors that recognize microbes in the blood and tissues, and either stimulate phagocytosis (e.g., mannose or opsonin receptors) or activate phagocytes not involved in ingestion (e.g., Toll-like receptors, TLRs). The effector mechanisms of innate immunity are often used to eliminate microbes, even in an adaptive immune response. Thus, the innate immune response can provide signals that function in concert with antigen to stimulate the proliferation and differentiation of antigen-specific (adaptive) T and B lymphocytes.

[0010] Stimulators of the innate immune response have been described. U.S. patent application Ser. No. 11/830,622 (Publication No. 2008/0170966) describes methods of attenuating respiratory infection by inhalation of a microbial lysate that stimulates innate immunity. U.S. patent application Ser. No. 10/972,062 (Publication No. 2009/0318337) describes methods of activating innate immunity by administering proteosome-based immunoactive compositions to a subject. U.S. patent application Ser. No. 12/556,759 (Publication No. 2011/0105383) describes methods for stimulation of innate immune resistance to pathogens by administering a recombinant bacterial protein to a subject.

[0011] This background information is provided for the purpose of making known information believed by the applicant to be of possible relevance to the present invention. No admission is necessarily intended, nor should be construed, that any of the preceding information constitutes prior art against the present invention.

SUMMARY OF THE INVENTION

[0012] An object of the present invention is to provide uses of papaya mosaic virus compositions for stimulation of the innate immune response. In accordance with one aspect of the invention, there is provided a use of a composition comprising Papaya Mosaic Virus (PapMV) or PapMV virus-like particles (VLPs) to stimulate an innate immune response in a subject and thereby prevent, or decrease the severity of, a microbial infection in the subject.

[0013] In accordance with another aspect of the invention, there is provided a use of a composition comprising Papaya Mosaic Virus (PapMV) or PapMV virus-like particles (VLPs) in the manufacture of a medicament to stimulate an innate immune response in a subject and thereby prevent, or decrease the severity of, a microbial infection in the subject.

[0014] In accordance with another aspect of the invention, there is provided a use of a composition comprising Papaya Mosaic Virus (PapMV) or PapMV virus-like particles (VLPs) to protect a subject against infection with a pathogen, wherein the composition stimulates the innate immune response in the subject.

[0015] In accordance with another aspect of the invention, there is provided a use of a composition comprising Papaya Mosaic Virus (PapMV) or PapMV virus-like particles (VLPs) in the manufacture of a medicament to protect a subject against infection with a pathogen, wherein the composition stimulates the innate immune response in the subject.

[0016] In accordance with another aspect of the invention, there is provided a use of a composition comprising Papaya Mosaic Virus (PapMV) or PapMV virus-like particles (VLPs) to treat a chronic or recurrent microbial infection in a subject.

[0017] In accordance with another aspect of the invention, there is provided a use of a composition comprising Papaya Mosaic Virus (PapMV) or PapMV virus-like particles (VLPs) in the manufacture of a medicament to treat a chronic or recurrent microbial infection in a subject.

[0018] In accordance with another aspect of the invention, there is provided a method of stimulating an innate immune response in a subject comprising administering to the subject an effective amount of a composition comprising papaya mosaic virus (PapMV) or PapMV virus-like particles (VLPs), thereby preventing, or decreasing the severity of, a microbial infection in the subject.

[0019] In accordance with another aspect of the invention, there is provided a method of protecting a subject against microbial infection comprising administering to the subject an effective amount of a composition comprising papaya mosaic virus (PapMV) or PapMV virus-like particles (VLPs), wherein the composition stimulates the innate immune response in the subject.

[0020] In accordance with another aspect of the invention, there is provided a method of treating a chronic or recurrent infection comprising administering to a subject having a chronic microbial infection a composition comprising Papaya Mosaic Virus (PapMV) or PapMV virus-like particles (VLPs).

[0021] In accordance with another aspect of the invention, there is provided a kit comprising a container having contained therein a pharmaceutical composition comprising papaya mosaic virus (PapMV) or PapMV virus-like particles (VLPs), the container adapted to deliver the pharmaceutical composition by an intranasal, pulmonary or vaginal route.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] These and other features of the invention will become more apparent in the following detailed description in which reference is made to the appended drawings.

[0023] FIG. **1** presents (A) the amino acid sequence of the wild-type PapMV coat protein (SEQ ID NO:1) and (B) the nucleotide sequence of the wild-type PapMV coat protein (SEQ ID NO:2).

[0024] FIG. **2** presents (A) the amino acid sequence of the modified PapMV coat protein CPAN5 (SEQ ID NO:3), and (B) the amino acid sequence of modified PapMV coat protein PapMV CPsm (SEQ ID NO:4).

[0025] FIG. **3** presents results demonstrating that PapMV VLPs induce an anti-viral response that controls influenza infection, (A) weight loss of Balb/C mice (10 per group) treated intranasally with 30 or 75 μg of PapMV VLPs or control buffer (PBS) and challenged with 100 pfu of influenza virus strain WSN/33, (B) symptoms developed in the mice during infection (Symptoms: 0, No symptoms. 1, Lightly spiked fur, slightly curved back. 2, Spiked fur, curved back. 3, Spiked fur, curved back, difficulty in moving and mild dehydration. 4, Spiked fur, curved back, difficulty in moving, severe dehydration, closed eyes and ocular secretion), and (C) survival rate of the infected mice.

[0026] FIG. **4** presents results demonstrating that PapMV VLPs induce an anti-viral response that controls influenza infection, (A) weight loss of Balb/C mice (10 per group) treated intranasally with 60 μg of PapMV VLPs or control buffer (PBS) and challenged with 200 pfu of influenza virus strain WSN/33, (B) symptoms developed in the mice during infection (Symptoms: as for FIG. **3**), and (C) survival rate of the infected mice.

[0027] FIG. **5** presents results demonstrating that PapMV VLPs induce an anti-viral response that controls influenza infection, (A) depicts the weight loss of Balb/C mice (10 per group) treated intranasally with PapMV VLPs containing ssRNA, 60 μg PapMV VLPs containing poly I:C, 3 μg ssRNA, 3 μg poly I:C, 60 μg of PapMV CP monomers or control buffer (Tris HCl 10 mM pH 8) and challenged with 200 pfu of influenza virus strain WSN/33, (B) presents a summary of the symptoms developed in the mice during infection (Symptoms: as for FIG. **3**).

[0028] FIG. **6** presents graphs indicating the presence of IP-10 (A) and IL-9 (B) in bronchoalveolar lavage of Balb/C mice treated intranasally with PapMV VLPs (60 μg), Pam3CSK4 (15 μg) or control buffer (Tris HCl 10 mM pH 8). Each point corresponds to the level of cytokines detected in each mouse. Also shown is the amount of IP-10 or IL-9 present in nasopharyngeal lavage ("LNP") from the mice.

[0029] FIG. **7** presents graphs indicating the presence of (A) MIP-1α, (B) MIP-1β, (C) MIP-2, (D) KC, (E) TNF-α, (F) RANTES, (G) VEGF, (H) MCP-1, (I) IP-10, (J) IL-17, (K) IL-13, (L) IL-12 (p70), (M) IL-9, (N) IL-6, (O) IL-1α, (P) IL-1β, (Q) GM-CSF and (R) G-CSF in bronchoalveolar lavage of Balb/C mice treated intranasally with one or two treatments of PapMV VLPs (60 μg) or with control buffer (Tris HCl 10 mM pH 8). Each point corresponds to the level of cytokines detected in each mouse.

[0030] FIG. **8** presents graphs depicting compilation of (A) CD86 and (B) CD69 expression in DCs, $CD8^+$ T cells and B cells of C57BL/6, TLR7 knockout (KO), MYD88 KO and IRF5/7 KO mice 24 h after PapMV VLP ssRNA (100 ng) or PBS immunization. Results were analyzed by FACS and are presented as a ratio of the Mean Fluorescence Intensity (MFI) of the analyzed sample on the PBS sample.

[0031] FIG. **9** presents graphs depicting compilation of flow cytometry analysis of (A) CD69, (B) MHC-I and (C) CD86 expression 24 h after immunization of C57BL/6 mice with PapMV PapMV VLP ssRNA with or without treatment with an anti-BST2 antibody. ***$p<0.001$, **$p<0.01$, *$p<0.05$, NS: not significant.

[0032] FIG. **10** presents graphs depicting (A) evaluation by ELISA of the kinetics of production of IFN-α in serum and (B) spleen of C57BL/6 mice following immunization with 100 μg PapMV VLP ssRNA, and (C) ELISA quantification of serum IFN-α in C57BL/6 and different knockout mice 6 h post-immunization with 100 ng PapMV VLP ssRNA or PBS.

[0033] FIG. **11** presents graphs depicting a compilation of CD86, MHC-I and CD69 expression in (A) B lymphocytes and (B) dendritic cells from spleens of C57BL/6 and IFNAR KO mice 24 h after immunization with PapMV VLP ssRNA or PBS, and (C) quantification by ELISA of antibody against PapMV VLP ssRNA in serum of C57BL/6 and IFNAR KO mice at different time points after PapMV VLP ssRNA immunization.

[0034] FIG. **12** presents a graph depicting the viral kinetics of LCMV clone 13 in blood of C57BL/6 mice treated with 100 μg PapMV VLP ssRNA (filled squares) or PBS (open circles) 6 hours before infection with 2×10^6 PFU LCMV clone 13 (titers are expressed in PFU per milliliter of blood; LOD: limit of detection).

[0035] FIG. **13** presents graphs depicting the viral titers in (A) spleen, (B) kidney, (C) liver and (D) brain of C57BL/6 and TLR7 knockout (KO) mice 15 days after infection with 2×10^6 PFU LCMV clone 13; mice were treated with 100 μg

PapMV VLP ssRNA, 100 μg R837 or PBS 6 hours before infection (titers are expressed in PFU per organ). LOD: limit of detection.

[0036] FIG. **14** presents graphs depicting the proportion of $CD8^+$ T cells producing (A) IFN-γ, (B) TNF-α and (C) both cytokines after GP33 restimulation of splenocytes isolated from mice immunized with 100 μg PapMV VLP ssRNA, 100 μg R837 or PBS 6 hours before infection with 2×10^6 pfu LCMV clone 13 and sacrificed 15 days post-infection; (D) amount of TFN-γ and (E) amount of TNF-α produced by $CD8^+$ T cells after GP33 restimulation, (F) Mean Fluorescence Intensity (MFT) of PD-1 expression in GP33 specific $CD8^+$ T lymphocytes, and (G) percentage of $DbGP33^+CD8^+$ $CD44^+$ in splenocytes.

[0037] FIG. **15** presents graphs depicting the viral titers in (A) spleen, (B) kidney, (C) brain and (D) liver of C57BL/6 mice 45 days after infection with 2×10^6 PFU LCMV clone 13; mice were treated with 100 μg PapMV VLP ssRNA or PBS 6 hours before infection (titers are expressed in PFU per organ). LOD: limit of detection.

[0038] FIG. **16** presents a chart depicting flow cytometry analysis of CD86 expression in human PBMCs ($CD14^+$ $CD11b^+$ cell population) 18 h after stimulation with PapMV VLP ssRNA (MFI: mean fluorescence intensity).

[0039] FIG. **17** presents a graph depicting survival of mice treated with 2 doses of PapMV VLP ssRNA at 2-week intervals prior to challenge with a sub-lethal dose of *Streptococcus pneumoniae*.

[0040] FIG. **18** presents graphs depicting viral load in mice chronically infected with LCMV and treated i.v. once/day with 100 μg PapMV VLP ssRNA (A) at day 1, 2, 3, 4 and 5 post-infection, and (B) at day 6 and 7 post-infection only (titers are expressed in PFU/mL).

[0041] FIG. **19** presents graphs depicting the viral titers in different organs of mice treated as described for FIG. **18** at day 15 (end of the experiment).

[0042] FIG. **20** presents a graph illustrating weight loss in mice treated once (1×), twice (2×), 5 times (5×) and 10 times (10×) at 1-week intervals with PapMV VLPs and challenged with the influenza WSN/33 virus 3 days after the last treatment.

[0043] FIG. **21** presents electron micrographs showing cells found in broncho-alveolar lavage (BAL) from mice treated with (A) buffer and (B) PapMV VLPs (neutrophils are circled), and (C) a graph depicting numbers of neutrophils found in the BAL.

[0044] FIG. **22** presents graphs depicting the IgG and IgG2a titers measured in the blood of mice immunized intranasally with PapMV VLPs combined with the trivalent inactivated flu vaccine (TIV), (A) total IgG titers after one immunization, (B) total IgG liters after two immunizations at 14-day intervals, and (C) IgG2a titers measured after two immunizations.

[0045] FIG. **23** presents graphs depicting the antibody titers measured in mice immunized as described for FIG. **22** after two immunizations, (A) IgA titers in the broncho-alveolar lavage (BAL), (B) total IgG titers in the BAL, and (C) IgA in the faeces.

[0046] FIG. **24** presents a graph showing weight loss in mice immunized as described for FIG. **22** and challenged at day 15 with $1LD_{50}$ of the influenza WSN/33 virus; weight loss was followed over a 14 day period.

[0047] FIG. **25** presents electron micrographs of (A) PapMV VLPs self-assembled with ssRNA. and (B) PapMV VLPs self-assembled with poly I:C (dsRNA).

[0048] FIG. **26** presents a graph demonstrating that PapMV VLPs interact with TLR-2 and CD14 in a human monocyte cell line (THP-1) and that this interaction is blocked with antibodies (Ac) to TLR-2 and CD 14.

[0049] FIG. **27** presents a flow chart outlining the steps for the preparation of in vitro assembled PapMV VLPs containing ssRNA in accordance with one embodiment of the invention (rCP=recombinant PapMV coat protein; SRT=synthetic RNA template; rVLP=recombinant VLP; Ec.prCP=*E. coli* containing plasmid encoding rCP; Ec. pSRT=*E. coli* containing plasmid encoding SRT).

[0050] FIG. **28** presents the sequence of a synthetic RNA template (SRT) [SEQ ID NO:5] used in one embodiment of the process outlined in FIG. **27**; all ATG codons have been mutated for TAA stop codons (bold), the first 16 nucleotides (underlined) are from the T7 transcription start site located within the pBluescript expression vector and the PapMV nucleation site for rVLP assembly is boxed.

[0051] FIG. **29** presents the sequence of a synthetic RNA template (SRT) [SEQ ID NO:6] used in one embodiment of the process outlined in FIG. **27**; the first G is the first nucleotide of the transcript; all ATG codons have been mutated for TAA stop codons (bold); the first 1500 nucleotides of the sequence are identical to SEQ ID NO:5.

DETAILED DESCRIPTION OF THE INVENTION

[0052] The present invention provides for compositions comprising a papaya mosaic virus (PapMV) moiety (referred to herein as "PapMV compositions") and their use for stimulation of the innate immune response. The PapMV moiety comprised by the PapMV compositions may be papaya mosaic virus or PapMV virus-like particles (VLPs). While the ability of PapMV to activate the innate immune response has been noted previously as contributing to the adjuvant effect of PapMV (Acosta-Ramirez et al., 2008, ibid.), it is demonstrated herein that when administered alone, PapMV and PapMV VLPs unexpectedly are also capable of stimulating an innate immune response that is sufficiently strong to result in protective and/or therapeutic effects. Moreover, the PapMV compositions are capable of stimulating an immune response at mucosal surfaces.

[0053] In certain embodiments, therefore, the present invention provides for the use of PapMV compositions to stimulate a protective non-specific immune response in an animal and thus provide protection against subsequent pathogen challenge. As demonstrated herein, the PapMV compositions are capable of stimulating a rapid innate immune response and can provide protection against pathogen infection for several days. In certain embodiments, the present invention provides for the use of PapMV compositions to protect a subject from potential infection by a pathogen that gains access to the body via mucosal membranes. For example, the PapMV composition may be administered alone in order to stimulate the innate immune response or may be administered in combination with one or more antigens and act as a mucosal adjuvant that allows for the generation of an effective mucosal immune response. In accordance with certain embodiments of the invention, therefore, the PapMV compositions are administered via a mucosal route and elicit a protective effect within the mucosa and/or in the respiratory

system. In certain embodiments of the invention, the pathogen is one or more of a viral pathogen, a bacterial pathogen or a fungal pathogen.

[0054] Certain embodiments of the invention provide for the use of PapMV compositions to treat an established infection, for example, an infection with a viral pathogen, a bacterial pathogen or a fungal pathogen. In some embodiments, PapMV compositions may be used to treat an infection at a mucosal surface, for example, in the lungs, intestines or genitourinary system.

[0055] Some embodiments of the invention provide for the use of PapMV compositions to decrease the viral load in a subject with a chronic viral infection and thus assist with management and/or clearance of the infection. Combination therapies using PapMV compositions and conventional therapies for chronic infection are also provided in some embodiments. In some embodiments, such combination therapies may, for example, result in one or more of an improved efficacy of the conventional therapy, a decrease in the dosage amount of the conventional therapy required to reach a predetermined endpoint, a decrease in the duration of treatment, a decrease in side-effects associated with the conventional therapy, or the like. Certain embodiments of the invention provide for the use of PapMV compositions, alone or in combination with a conventional therapy, to treat immune exhaustion in a subject with a chronic infection.

[0056] Typically, the compositions comprise the PapMV or PapMV VLPs and a suitable carrier or diluent, but compositions consisting of just the PapMV or PapMV VLPs in a suitable form for administration to a subject (for example, freeze dried or lyophilized) remain an alternative option in some embodiments. Compositions comprising mixtures of PapMV and PapMV VLPs are also contemplated in certain embodiments.

DEFINITIONS

[0057] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0058] As used herein, the term "about" refers to approximately a +/−10% variation from a given value. It is to be understood that such a variation is always included in any given value provided herein.

[0059] The use of the word "a" or "an" when used herein in conjunction with the term "comprising" may mean "one," but it is also consistent with the meaning of "one or more," "at least one" and "one or more than one."

[0060] As used herein, the words "comprising" (and grammatical variations thereof, such as "comprise" and "comprises"), "having" (and grammatical variations thereof, such as "have" and "has"), "including" (and grammatical variations thereof, such as "includes" and "include") or "containing" (and grammatical variations thereof, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0061] "Naturally occurring," as used herein, as applied to an object, refers to the fact that an object can be found in nature. For example, an organism, or a polypeptide or polynucleotide sequence that is present in an organism that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally occurring.

[0062] The terms "attenuate," "inhibit," "prevent" and grammatical variations thereof, as used herein, refer to a measurable decrease in a given parameter or event.

[0063] The term "vaccine," as used herein, refers to a composition capable of producing a beneficial immune response when administered to a subject.

[0064] The term "pathogen," as used herein, refers to an organism capable of causing a disease or disorder in a host including, but not limited to, bacteria, viruses, protozoa, fungi and parasites.

[0065] The term "subject" or "patient" as used herein refers to an animal in need of treatment.

[0066] The term "animal," as used herein, refers to both human and non-human animals, including, but not limited to, mammals, birds and fish, and encompasses domestic, farm, zoo, laboratory and wild animals, such as, for example, cows, pigs, horses, goats, sheep or other hoofed animals, dogs, cats, chickens, ducks, non-human primates, guinea pigs, rabbits, ferrets, rats, hamsters and mice.

[0067] Administration of PapMV compositions "in combination with" one or more further therapeutic agents is intended to include simultaneous (concurrent) administration and consecutive administration. Consecutive administration is intended to encompass various orders of administration of the therapeutic agent(s) and the composition of the invention to the subject with administration of the therapeutic agent(s) and the composition being separated by a defined time period that may be short (for example in the order of minutes) or extended (for example in the order of days or weeks).

[0068] The term "substantially identical," as used herein in relation to a nucleic acid or amino acid sequence indicates that, when optimally aligned, for example using the methods described below, the nucleic acid or amino acid sequence shares at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity with a defined second nucleic acid or amino acid sequence (or "reference sequence"). "Substantial identity" may be used to refer to various types and lengths of sequence, such as full-length sequence, functional domains, coding and/or regulatory sequences, promoters, and genomic sequences. Percent identity between two amino acid or nucleic acid sequences can be determined in various ways that are within the skill of a worker in the art, for example, using publicly available computer software such as Smith Waterman Alignment (Smith, T. F. and M. S. Waterman (1981) *J Mol Biol* 147:195-7); "BestFit" (Smith and Waterman, *Advances in Applied Mathematics,* 482-489 (1981)) as incorporated into GeneMatcher Plus™, Schwarz and Dayhof (1979) *Atlas of Protein Sequence and Structure*, Dayhof, M. O., Ed pp 353-358; BLAST program (Basic Local Alignment Search Tool (Altschul, S. F., W. Gish, et al. (1990) *J Mol Biol* 215: 403-10), and variations thereof including BLAST-2, BLAST-P, BLAST-N, BLAST-X, WU-BLAST-2, ALIGN, ALIGN-2, CLUSTAL, and Megalign (DNASTAR) software. In addition, those skilled in the art can determine appropriate parameters for measuring alignment, including algorithms needed to achieve maximal alignment over the length of the sequences being compared. In general, for amino acid sequences, the length of comparison sequences will be at least 10 amino acids. One skilled in the art will understand that the actual length will depend on the overall length of the sequences being compared and may be at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least

110, at least 120, at least 130, at least 140, at least 150, or at least 200 amino acids, or it may be the full-length of the amino acid sequence. For nucleic acids, the length of comparison sequences will generally be at least 25 nucleotides, but may be at least 50, at least 100, at least 125, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 550, or at least 600 nucleotides, or it may be the full-length of the nucleic acid sequence.

[0069] The terms "corresponding to" or "corresponds to" indicate that a nucleic acid sequence is identical to all or a portion of a reference nucleic acid sequence. In contradistinction, the term "complementary to" is used herein to indicate that the nucleic acid sequence is identical to all or a portion of the complementary strand of a reference nucleic acid sequence. For illustration, the nucleic acid sequence "TATAC" corresponds to a reference sequence "TATAC" and is complementary to a reference sequence "GTATA." The terms "corresponding to" and "corresponds to" when used herein to cross-reference a DNA and RNA sequence indicate that the DNA sequence is identical to all of a portion of the reference RNA sequence (or vice versa), however, the DNA sequence will contain thymine (T) residues at positions corresponding to uracil (U) residues in the RNA sequence. Thus, for illustration, the DNA sequence "TATAC" corresponds to an RNA reference sequence "UAUAC."

[0070] It is contemplated that any embodiment discussed herein can be implemented with respect to any method or composition of the invention, and vice versa. Furthermore, compositions and kits of the invention can be used to achieve methods of the invention.

PapMV Moiety

[0071] The PapMV moiety in accordance with the present invention may be either PapMV or PapMV VLPs. In certain embodiments of the invention, the compositions comprising VLPs may also comprise minor amounts of multimerised PapMV coat protein in the form of discs.

[0072] PapMV is known in the art and can be obtained, for example, from the American Type Culture Collection (ATCC) as ATCC No. PV204™.

[0073] PapMV VIPs are formed from recombinant PapMV coat proteins that have multimerised and self-assembled to form a VLP. When assembled, each VLP comprises a long helical array of coat protein subunits. The wild-type virus comprises over 1200 coat protein subunits and is about 500 nm in length. PapMV VLPs that are either shorter or longer than the wild-type virus can still, however, be effective. In certain embodiments of the present invention, a VLP may comprise at least 40 coat protein subunits. In some embodiments, a VLP may comprise between about 40 and about 1600 coat protein subunits. VLPs comprising a greater number of coat proteins are also contemplated. In some embodiments, a VLP may be at least 40 nm in length. In some embodiments, a VLP may be between about 40 nm and about 600 nm in length. Certain embodiments of the invention contemplate VLPs of greater than 600 nm in length.

[0074] The VLPs in accordance with the present invention can be prepared from a plurality of recombinant coat proteins having identical amino acid sequences, such that the final VLP when assembled comprises identical coat protein subunits, or the VLP can be prepared from a plurality of recombinant coat proteins having different amino acid sequences, such that the final VLP when assembled comprises variations in its coat protein subunits.

[0075] The PapMV coat protein used to prepare the VLPs can be the entire PapMV coat protein, or part thereof, or it can be a genetically modified version of the wild-type PapMV coat protein, for example, comprising one or more amino acid deletions, insertions, replacements and the like, provided that the coat protein retains the ability to self-assemble into a VLP. The amino acid sequence of the wild-type PapMV coat (or capsid) protein is known in the art (see, Sit, et al., 1989, *J. Gen. Virol.*, 70:2325-2331, and GenBank Accession No. NP_044334.1) and is provided herein as SEQ ID NO:1 (see FIG. **1**A). Variants of this sequence are known, for example, the coat proteins of Mexican isolates of PapMV described by Noa-Carrazana & Silva-Rosales (2001, *Plant Science*, 85:558) have 88% sequence identity with SEQ ID NO:1. The nucleotide sequence of the PapMV coat protein is also known in the art (see, Sit, et al., *ibid.*, and GenBank Accession No. NC_001748 (nucleotides 5889-6536)) and is provided herein as SEQ ID NO:2 (see FIG. **1**B).

[0076] As noted above, the amino acid sequence of the PapMV coat protein need not correspond precisely to the parental (wild-type) sequence, i.e. it may be a "variant sequence." For example, the PapMV coat protein may be mutagenized by substitution, insertion or deletion of one or more amino acid residues so that the residue at that site does not correspond to the parental (reference) sequence. One skilled in the art will appreciate, however, that such mutations will not be extensive and will not dramatically affect the ability of the recombinant PapMV CP to assemble into VLPs. The ability of a variant version of the PapMV coat protein to assemble into VLPs can be assessed, for example, by electron microscopy following standard techniques, such as the exemplary methods described in U.S. patent application Ser. No. 11/556,678.

[0077] Recombinant PapMV CPs prepared using fragments of the wild-type coat protein that retain the ability to multimerise and assemble into a VLP (i.e. are "functional" fragments) are, therefore, also contemplated by the present invention. For example, a fragment may comprise a deletion of one or more amino acids from the N-terminus, the C-terminus, or the interior of the protein, or a combination thereof. In general, functional fragments are at least 100 amino acids in length. In one embodiment of the present invention, functional fragments are at least 150 amino acids, at least 160 amino acids, at least 170 amino acids, at least 180 amino acids, and at least 190 amino acids in length. Deletions made at the N-terminus of the wild-type protein should generally delete fewer than 13 amino acids in order to retain the ability of the protein to self-assemble.

[0078] In certain embodiments of the present invention, when a recombinant coat protein comprises a variant sequence, the variant sequence is at least about 70% identical to the reference sequence. In some embodiments, the variant sequence is at least about 75% identical to the reference sequence. In other embodiments, the variant sequence is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 97% identical, and at least about 98% identical to the reference sequence. In certain embodiments, the reference amino acid sequence is SEQ ID NO:1 (FIG. **1**A).

[0079] In certain embodiments of the present invention, the PapMV CP used to prepare the recombinant PapMV CP is a genetically modified (i.e. variant) version of the PapMV coat protein. In some embodiments, the PapMV coat protein has been genetically modified to delete amino acids from the N-

or C-terminus of the protein and/or to include one or more amino acid substitutions. In some embodiments, the PapMV coat protein has been genetically modified to delete between about 1 and about 10 amino acids from the N- or C-terminus of the protein, for example between about 1 and about 5 amino acids.

[0080] In certain embodiments, the PapMV coat protein has been genetically modified to remove one of the two methionine codons that occur proximal to the N-terminus of the wild-type protein (i.e. at positions 1 and 6 of SEQ ID NO:1) and can initiate translation. Removal of one of the translation initiation codons allows a homogeneous population of proteins to be produced. The selected methionine codon can be removed, for example, by substituting one or more of the nucleotides that make up the codon such that the codon codes for an amino acid other than methionine, or becomes a nonsense codon. Alternatively all or part of the codon, or the 5' region of the nucleic acid encoding the protein that includes the selected codon, can be deleted. In some embodiments of the present invention, the PapMV coat protein has been genetically modified to delete between 1 and 5 amino acids from the N-terminus of the protein. In some embodiments, the genetically modified PapMV coat protein has an amino acid sequence substantially identical to SEQ ID NO:3 (FIG. **2**A) and may optionally comprise a histidine tag of up to 6 histidine residues. In some embodiments, the PapMV coat protein has been genetically modified to include additional amino acids (for example between about 1 and about 8 amino acids) at the C-terminus that result from the inclusion of one or more specific restriction enzyme sites into the encoding nucleotide sequence. In some embodiments, the PapMV coat protein has an amino acid sequence substantially identical to SEQ ID NO:4 (FIG. **2**B) with or without the histidine tag.

[0081] When the recombinant PapMV coat protein is prepared using a variant PapMV CP sequence that contains one or more amino acid substitutions, these can be "conservative" substitutions or "non-conservative" substitutions. A conservative substitution involves the replacement of one amino acid residue by another residue having similar side chain properties. As is known in the art, the twenty naturally occurring amino acids can be grouped according to the physicochemical properties of their side chains. Suitable groupings include alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine and tryptophan (hydrophobic side chains); glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine (polar, uncharged side chains); aspartic acid and glutamic acid (acidic side chains) and lysine, arginine and histidine (basic side chains). Another grouping of amino acids is phenylalanine, tryptophan, and tyrosine (aromatic side chains). A conservative substitution involves the substitution of an amino acid with another amino acid from the same group. A non-conservative substitution involves the replacement of one amino acid residue by another residue having different side chain properties, for example, replacement of an acidic residue with a neutral or basic residue, replacement of a neutral residue with an acidic or basic residue, replacement of a hydrophobic residue with a hydrophilic residue, and the like.

[0082] In certain embodiments of the present invention, the variant sequence comprises one or more non-conservative substitutions. Replacement of one amino acid with another having different properties may improve the properties of the coat protein. For example, as previously described, mutation of residue 128 of the coat protein improves assembly of the protein into VLPs (Tremblay et al. 2006, *FEBS J.* 273:14-25). In some embodiments of the present invention, therefore, the coat protein comprises a mutation at residue 128 of the coat protein in which the glutamic acid residue at this position is substituted with a neutral residue. In some embodiments, the glutamic acid residue at position 128 is substituted with an alanine residue.

[0083] Substitution of the phenylalanine residue at position F13 of the wild-type PapMV coat protein with another hydrophobic residue has been shown to result in a higher proportion of VLPs being formed when the recombinant protein is expressed than when the wild-type protein sequence is used (Laliberté-Gagné, et al., 2008, *FEBS J.*, 275:1474-1484). In the context of the present invention, the following amino acid residues are considered to be hydrophobic residues suitable for substitution at the F13 position: Ile, Trp, Leu, Val, Met and Tyr. In some embodiments of the invention, the coat protein comprises a substitution of Phe at position 13 with Ile, Trp, Leu, Val, Met or Tyr. In some embodiments, the coat protein comprises a substitution of Phe at position 13 with Leu or Tyr.

[0084] In certain embodiments, mutation at position F13 of the CP may be combined with a mutation at position E128, a deletion at the N-terminus, or a combination thereof.

[0085] Likewise, the nucleic acid sequence encoding the PapMV coat protein used to prepare the recombinant PapMV CP need not correspond precisely to the parental reference sequence but may vary by virtue of the degeneracy of the genetic code and/or such that it encodes a variant amino acid sequence as described above. In certain embodiments of the present invention, therefore, the nucleic acid sequence encoding the variant coat protein is at least about 70% identical to the reference sequence. In some embodiments, the nucleic acid sequence encoding the variant coat protein is at least about 75% identical to the reference sequence. In other embodiments, the nucleic acid sequence encoding the variant coat protein is at least about 80%, at least about 85% or at least about 90% identical to the reference sequence. In certain embodiments, the reference nucleic acid sequence is SEQ ID NO:2 (FIG. **1**B).

Preparation of PapMV and PapMV VLPs

PapMV

[0086] PapMV is known in the art and can be obtained, for example, from the American Type Culture Collection (ATCC) as ATCC No. PV-204™. The virus can be maintained on, and purified from, host plants such as papaya (*Carica papaya*) and snapdragon (*Antirrhinum majus*) following standard protocols (see, for example, Erickson, J. W. & Bancroft, J. B., 1978, Virology 90:36-46).

PapMV VLPs

[0087] PapMV VLPs can be prepared by standard techniques known in the art (see, for example, Tremblay et al. 2006, ibid., and International Patent Application Nos. PCT/CA2007/002069, PCT/CA2008/000154 and PCT/CA2009/00636), as well as by in vitro assembly techniques as described herein.

[0088] Recombinant PapMV coat proteins for the preparation of PapMV VLPs can be readily prepared by standard genetic engineering techniques by the skilled worker provided with the sequence of the wild-type protein. Methods of

genetically engineering proteins are well known in the art (see, for example, Ausubel et al. (1994 & updates) *Current Protocols in Molecular Biology*, John Wiley & Sons, New York), as is the sequence of the wild-type PapMV coat protein (see, for example, SEQ ID NOs: 1 and 2).

[0089] For example, isolation and cloning of the nucleic acid sequence encoding the wild-type protein can be achieved using standard techniques (see, for example, Ausubel et al., *ibid*). For example, the nucleic acid sequence can be obtained directly from the PapMV by extracting RNA by standard techniques and then synthesizing cDNA from the RNA template (for example, by RT-PCR). PapMV can be purified from infected plant leaves that show mosaic symptoms by standard techniques.

[0090] The nucleic acid sequence encoding the coat protein is then inserted directly or after one or more subcloning steps into a suitable expression vector. One skilled in the art will appreciate that the precise vector used is not critical to the instant invention. Examples of suitable vectors include, but are not limited to, plasmids, phagemids, cosmids, bacteriophage, baculoviruses, retroviruses or DNA viruses. The coat protein can then be expressed and purified as described previously and below.

[0091] Alternatively, the nucleic acid sequence encoding the coat protein can be further engineered to introduce one or more mutations, such as those described above, by standard in vitro site-directed mutagenesis techniques well-known in the art. Mutations can be introduced by deletion, insertion, substitution, inversion, or a combination thereof, of one or more of the appropriate nucleotides making up the coding sequence. This can be achieved, for example, by PCR-based techniques for which primers are designed that incorporate one or more nucleotide mismatches, insertions or deletions. The presence of the mutation can be verified by a number of standard techniques, for example by restriction analysis or by DNA sequencing.

[0092] One of ordinary skill in the art will appreciate that the DNA encoding the coat protein can be altered in various ways without affecting the activity of the encoded protein. For example, variations in DNA sequence may be used to optimize for codon preference in a host cell used to express the protein, or may contain other sequence changes that facilitate expression.

[0093] One skilled in the art will understand that the expression vector may further include regulatory elements, such as transcriptional elements, required for efficient transcription of the DNA sequence encoding the coat or fusion protein. Examples of regulatory elements that can be incorporated into the vector include, but are not limited to, promoters, enhancers, terminators, and polyadenylation signals. Certain embodiments of the present invention, therefore, provide vectors comprising regulatory element operatively linked to a nucleic acid sequence encoding a genetically engineered coat protein. One skilled in the art will appreciate that selection of suitable regulatory elements is dependent on the host cell chosen for expression of the genetically engineered coat protein and that such regulatory elements may be derived from a variety of sources, including bacterial, fungal, viral, mammalian or insect genes.

[0094] In the context of the present invention, the expression vector may additionally contain heterologous nucleic acid sequences that facilitate the purification of the expressed protein. Examples of such heterologous nucleic acid sequences include, but are not limited to, affinity tags such as metal-affinity tags, histidine tags, avidin/streptavidin encoding sequences, glutathione-S-transferase (GST) encoding sequences and biotin encoding sequences. The amino acids encoded by the heterologous nucleic acid sequence can be removed from the expressed coat protein prior to use according to methods known in the art. Alternatively, the amino acids corresponding to expression of heterologous nucleic acid sequences can be retained on the coat protein if they do not interfere with its subsequent assembly into VLPs.

[0095] In one embodiment of the present invention, the coat protein is expressed as a histidine tagged protein. The histidine tag can be located at the carboxyl terminus or the amino tenninus of the coat protein.

[0096] The expression vector can be introduced into a suitable host cell or tissue by one of a variety of methods known in the art. Such methods can be found generally described in Ausubel et al. (*ibid.*) and include, for example, stable or transient transfection, lipofection, electroporation, and infection with recombinant viral vectors. One skilled in the art will understand that selection of the appropriate host cell for expression of the coat protein will be dependent upon the vector chosen. Examples of host cells include, but are not limited to, bacterial, yeast, insect, plant and mammalian cells. The precise host cell used is not critical to the invention. The coat proteins can be produced in a prokaryotic host (e.g. *E. coli, A. salmonicida* or *B. subtilis*) or in a eukaryotic host (e.g. *Saccharomyces* or *Pichia*; mammalian cells, e.g. COS, NIH 3T3, CHO, BHK, 293 or HeLa cells; insect cells or plant cells).

[0097] If desired, the coat proteins can be purified from the host cells by standard techniques known in the art (see, for example, in *Current Protocols in Protein Science*, ed. Coligan, J. E., et al., Wiley & Sons, New York, N.Y.) and sequenced by standard peptide sequencing techniques using either the intact protein or proteolytic fragments thereof to confirm the identity of the protein.

[0098] The recombinant coat proteins of the present invention are capable of self-assembly into VLPs. Assembly of the VLPs can take place, for example, in the host cell expressing the coat protein (see for example, Tremblay, et al., 2006, *FEBS J.*, 273:14-25), or it may take place in vitro, as described in more detail below (see also, International patent application Ser. No. _____ "Virus-Like Particles and Process for Preparing Same," Filed May 1, 2012, herein incorporated by reference in its entirety). In some embodiments, the VLPs comprise ssRNA. For example, VLPs that are prepared by expression of recombinant PapMV coat protein in *E. coli* and self-assembly of the coat protein in the bacteria may comprise bacterial ssRNA. Alternatively, for VLPs assembled in vitro, ssRNA may be added to the coat protein preparation prior to self-assembly. The ssRNA may be, for example, synthetic ssRNA, a naturally occurring ssRNA, a modified naturally occurring ssRNA, a fragment of a naturally occurring or synthetic ssRNA, or the like.

[0099] Typically, the ssRNA for in vitro assembly is at least about 100 nucleotides in length and up to about 5000 nucleotides in length, for example, at least about 250, 300, 350, 400, 450 or 500 nucleotides in length and up to about 5000, 4500, 4000 or 3500 nucleotides in length. In certain embodiments, the ssRNA for in vitro assembly is between about 500 and about 3000 nucleotides in length. In certain embodiments, the ssRNA for in vitro assembly is designed such that it does not include any ATG start codons in order to minimize the chances of in vivo transcription of the sequences, how-

ever, inclusion of ATG start codons is not excluded as in vivo transcription remains unlikely as the ssRNA is not capped. In certain embodiments, the ssRNA for in vitro assembly includes about 100 nucleotides from the 5'-end of the native PapMV RNA, which correspond to the putative packaging signal. ssRNA that does not include the putative packaging signal can also be assembled successfully. Non-limiting examples of ssRNA based on the PapMV genome that may be used in this regard are provided in FIGS. **28** and **29** [SEQ ID NOs: 5 and 6].

[0100] The VLPs can be isolated from host cells by standard techniques, such as those described in Tremblay, et al. (2006, *FEBS J.,* 273:14-25) and in the Examples. The VLPs can be further purified by standard techniques, such as chromatography, if desired to remove contaminating host cell proteins or other compounds, such as LPS.

[0101] When required, the VLPs can be separated from the other coat protein components by, for example, ultracentrifugation or gel filtration chromatography (for example, using Superdex G-200) to provide a substantially pure VLP preparation. In this context, by "substantially pure" it is meant that the preparation contains 70% or greater of VLPs, for example, 80% or 90% or greater.

[0102] In certain embodiments, PapMV VLPs comprise ssRNA and are prepared by in vitro assembly with a ssRNA template. Exemplary ssRNA template is shown in FIGS. **28** and **29** [SEQ ID NOs: 5 and 6], but one skilled in the art will appreciate that various other ssRNA would be suitable for this purpose, as described above. An exemplary method for in vitro assembly is shown in FIG. **28** and described in Example 18. It will be recognized that various alterations may be made to this method and still provide VLPs. For example, expression of the recombinant coat protein and ssRNA template can be effected in other host cells, such as *Pichia pastoris*, and the assembly reaction may be conducted at temperatures ranging from 2° C. to 37° C. with various ratios of protein:ssRNA. In general, a protein:ssRNA ratio between about 1:1 and about 50:1 by weight may be used, for example, between about 5:1 and about 50:1, between about 5:1 and about 40:1, or between about 10:1 and about 40:1 by weight.

Characteristics of Recombinant PapMV Coat Proteins

[0103] Recombinant coat proteins can be analysed for their ability to self-assemble into VLPs by standard techniques. For example, by visualising the purified protein by electron microscopy (see, for example, Tremblay, et al., 2006, *FEBS J.,* 273:14-25). In addition, ultracentrifugation may be used to isolate VLPs as a pellet, while leaving smaller aggregates (20-mers and less) in the supernatant, and circular dichroism (CD) spectrophotometry may be used to compare the secondary structure of the recombinant or modified proteins with the WT virus (see, for example, Tremblay et al., *ibid.*).

[0104] Stability of the VLPs and of PapMV can be determined if desired by techniques known in the art, for example, by SDS-PAGE and proteinase K degradation analyses. According to some embodiments of the present invention, the PapMV VLPs are stable at elevated temperatures and can be stored easily at room temperature.

Evaluation of Efficacy

[0105] The efficacy of the PapMV compositions in stimulating the innate immune response and producing a protective or therapeutic effect can be evaluated by standard techniques known in the art. For example, for protective effects, challenge studies can be conducted. Such studies involve the inoculation of groups of test animals (such as mice) with the PapMV composition by standard techniques. Control groups comprising non-inoculated animals and/or animals inoculated with a known stimulator of the innate immune response, or other positive control, are set up in parallel. After an appropriate period of time post-vaccination, the animals are challenged with the pathogen of interest. The animals are monitored for development of other conditions associated with infection including, for example, body temperature, weight, and the like. In certain cases, for example when the antigen is from certain strains of influenza or other pathogens associated with mortality, survival is also a suitable marker. The extent of infection can also be assessed, if desired, by measurement of viral titers using standard techniques after sacrifice of the animal.

[0106] For therapeutic studies, similar methods can be employed using standard animal models of infection and with the animals being treated with the PapMV composition at an appropriate time post-infection.

[0107] In addition, blood samples collected from the animals at various time intervals, for example pre-inoculation, post-inoculation and/or post-challenge, can be analyzed for cytokine and/or chemokine induction if desired using standard tests known in the art.

[0108] Other standard techniques may also be employed to assess the compositions, including, for example, evaluation of efficacy in combination with conventional prophylactic or therapeutic drugs or vaccines in various animal models of infection and disease known in the art.

Pharmaceutical Compositions and Administration

[0109] The present invention provides for pharmaceutical compositions comprising the PapMV moiety and one or more pharmaceutically acceptable carriers, diluents and/or excipients. If desired, other active ingredients may be included in the compositions, for example, additional immune stimulating compounds, standard therapeutics, vaccines or the like.

[0110] The pharmaceutical compositions can be formulated for administration by a variety of routes. For example, the compositions can be formulated for oral, topical, rectal, nasal or parenteral administration or for administration by inhalation or spray. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrathecal, intrasternal injection or infusion techniques. Intranasal administration to the subject includes administering the composition to the mucous membranes of the nasal passage or nasal cavity of the subject.

[0111] In some embodiments, the pharmaceutical compositions are formulated for mucosal administration. Mucosal administration may include, for example, oral, intranasal, aerosol, rectal or vaginal administration. The preparations for mucosal administration include transdermal devices, aerosols, creams, lotions or powders pending on the mucosal site. In certain embodiments, the pharmaceutical compositions are formulated for intranasal or pulmonary administration. In some embodiments, the pharmaceutical compositions are formulated for rectal or vaginal administration.

[0112] The pharmaceutical compositions comprise an effective amount of the PapMV moiety. The effective amount for a given indication can be estimated initially, for example, in animal models, usually in rodents, rabbits, dogs, pigs or primates. The animal model may also be used to determine

the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in the animal to be treated, including humans. In certain embodiments of the present invention, the unit dose comprises between about 10 μg to about 10 mg of protein, for example, between about 10 μg to about 5 mg of protein, or between about 40 μg to about 2 mg of protein. One or more doses may be used to immunise the subject, and these may be administered on the same day or over the course of several days or weeks.

[0113] Compositions formulated as aqueous suspensions may contain the PapMV moiety in admixture with one or more suitable excipients, for example, with suspending agents, such as sodium carboxymethylcellulose, methyl cellulose, hydropropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, hydroxypropyl-β-cyclodextrin, gum tragacanth and gum acacia; dispersing or wetting agents such as a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example, polyoxyethyene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example, hepta-decaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol for example, polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example, polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxy-benzoate, one or more colouring agents, one or more flavouring agents or one or more sweetening agents, such as sucrose or saccharin.

[0114] In certain embodiments, the pharmaceutical compositions may be formulated as oily suspensions by suspending the PapMV moiety in a vegetable oil, for example, arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example, beeswax, hard paraffin or cetyl alcohol. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid.

[0115] In certain embodiments, the pharmaceutical compositions may be formulated as a dispersible powder or granules, which can subsequently be used to prepare an aqueous suspension by the addition of water. Such dispersible powders or granules provide the PapMV moiety in admixture with one or more dispersing or wetting agents, suspending agents and/or preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example, colouring agents, can also be included in these compositions.

[0116] Pharmaceutical compositions of the invention may also be formulated as oil-in-water emulsions in some embodiments. The oil phase can be a vegetable oil, for example, olive oil or arachis oil, or a mineral oil, for example, liquid paraffin, or it may be a mixture of these oils. Suitable emulsifying agents for inclusion in these compositions include naturally-occurring gums, for example, gum acacia or gum tragacanth; naturally-occurring phosphatides, for example, soy bean, lecithin; or esters or partial esters derived from fatty acids and hexitol, anhydrides, for example, sorbitan monoleate, and condensation products of the said partial esters with ethylene oxide, for example, polyoxyethylene sorbitan monoleate.

[0117] In certain embodiments, the pharmaceutical compositions may be formulated as a sterile injectable aqueous or oleaginous suspension according to methods known in the art and using suitable one or more dispersing or wetting agents and/or suspending agents, such as those mentioned above. The sterile injectable preparation can be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Acceptable vehicles and solvents that can be employed include, but are not limited to, water, Ringer's solution, lactated Ringer's solution and isotonic sodium chloride solution. Other examples include, sterile, fixed oils, which are conventionally employed as a solvent or suspending medium, and a variety of bland fixed oils including, for example, synthetic mono- or diglycerides. Fatty acids such as oleic acid can also be used in the preparation of injectables.

[0118] Optionally the pharmaceutical compositions may contain preservatives such as antimicrobial agents, anti-oxidants, chelating agents, and inert gases, and/or stabilizers such as a carbohydrate (e.g. sorbitol, mannitol, starch, sucrose, glucose, or dextran), a protein (e.g. albumin or casein), or a protein-containing agent (e.g. bovine serum or skimmed milk) together with a suitable buffer (e.g. phosphate buffer). The pH and exact concentration of the various components of the composition may be adjusted according to well-known parameters.

[0119] Sterile compositions can be prepared for example by incorporating the PapMV moiety in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile compositions, some exemplary methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0120] Contemplated for use in certain embodiments of the invention are various mechanical devices designed for pulmonary or intranasal delivery of therapeutic products, including but not limited to, nebulizers, metered dose inhalers, powder inhalers and nasal spray devices, all of which are familiar to those skilled in the art.

[0121] Metered dose inhalers typically use a propellant gas and require actuation during inspiration. Dry powder inhalers use breath-actuation of a mixed powder. Nebulizers produce aerosols from solutions, while metered dose inhalers, dry powder inhalers, and the like generate small particle aerosols.

[0122] Some specific examples of commercially available devices suitable for the practice of this invention are the ULTRAVENT® nebulizer (Mallinckrodt, Inc., St. Louis, Mo.), the ACORN II® nebulizer (Marquest Medical Products, Englewood, Colo.), the MISTY-NEB® nebulizer (Allegiance, McGraw Park, Ill.), the AEROECLIPSE® nebulizer (Trudell Medical International, Canada), the Accuspray™ nasal spray device (Becton Dickinson), the Mucosal Atomization Device (MAD300) (Wolfe Tory Medical), the OptiNose device (OptiNose, Oslo, Norway), the Nektar DPI system (Nektar Therapeutics, Inc., San Carlos, Calif.), the AERx pulmonary drug delivery system (Aradigm Corporation, Hayward, Calif.), the Spiros® device (Dura Pharmaceuticals), and the Respimat® device (Boehringer Ingelheim).

[0123] All such devices require the use of formulations suitable for the dispensing of the PapMV moiety. Typically, each formulation is specific to the type of device employed

and may involve the use of an appropriate propellant material, in addition to the usual diluents, adjuvants and/or carriers useful in therapy as would be understood by a worker skilled in the art. Also, the use of liposomes, microcapsules or microspheres, inclusion complexes, or other types of carriers is contemplated.

[0124] Thus, in some embodiments, the invention provides for pharmaceutical compositions that are formulated for delivery via an intranasal or pulmonary route in, for example, lyophilized powder form, in an aerosolized liquid form, or in a gel form. These routes of administration can also allow for easy administration in the event of the need for mass distribution.

[0125] Formulations suitable for use with a nebulizer, either jet or ultrasonic, will typically comprise the PapMV moiety in an aqueous medium at a suitable concentration, for example, about 0.01 mg to 25 mg, or about 0.1 mg to 10 mg, of protein per mL of solution. The formulation may also include a buffer and a simple sugar (for example, for protein stabilization and regulation of osmotic pressure), and/or human serum albumin ranging in concentration from about 0.1 to about 10 mg/ml. Examples of buffers that may be used include, but are not limited to, sodium acetate, citrate and glycine. Typically, the buffer will have a composition and molarity suitable to adjust the solution to a pH in the range of 3 to 9. Generally, buffer molarities of from 1 mM to 50 mM are suitable for this purpose. Examples of excipients, usually in amounts ranging from about 1% to about 90% by weight (for example, from about 1% to about 50% by weight, or about 5% to about 30% by weight) of the formulation include, but are not limited to, monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; alditols, such as mannitol, xylitol, xylose, maltitol, lactitol, xylitol sorbitol (glucitol), sorbitose, pyranosyl sorbitol, myoinositol and the like; and glycine, $CaCl_2$, hydroxyectoine, ectoine, gelatin, di-myo-inositol phosphate (DIP), cyclic 2,3-diphosphoglycerate (cDPG), 1,1-di-glycerol phosphate (DGP), β-mannosylglycerate (firoin), β-mannosylglyceramide (firoin A), proline betaine and/or derivatives, as well as combinations thereof.

[0126] The nebulizer formulation may also contain a surfactant to reduce or prevent surface induced aggregation of the composition components caused by atomization of the solution in forming the aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbitan fatty acid esters. Amounts will generally range between about 0.001% and about 4% by weight of the formulation. A non-limiting example of a surfactant for this purpose is polyoxyethylene sorbitan monooleate.

[0127] In certain embodiments, the pharmaceutical compositions can be delivered in powder form using, for example, a metered dose inhaler device. This powder may be produced by lyophilization and may also contain a stabilizer such as human serum albumin (HSA). Additionally, one or more of the following may be added as an excipient to the composition, if necessary, to enhance one or more features (for example, to facilitate dispersal of the powder from a device, to increase the shelf-life of the composition, or to improve the stability of the composition during lyophilization): monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; alditols, such as mannitol, xylitol, xylose, maltitol, lactitol, xylitol sorbitol (glucitol), sorbitose, pyranosyl sorbitol, myoinositol and the like; and glycine, $CaCl_2$, hydroxyectoine, ectoine, gelatin, di-myo-inositol phosphate (DIP), cyclic 2,3-diphosphoglycerate (cDPG), 1,1-di-glycerol phosphate (DGP), β-mannosylglycerate (firoin), β-mannosylglyceramide (firoin A), proline, betaine and/or derivatives as well as combinations thereof. The amount added to the composition can range from about 0.01% to 200% (w/w), for example, from about 1% to 50% (w/w), or from about 5% to 30% (w/w) of the protein present. Such formulations are then lyophilized and milled to the desired particle size. Typically, the particles of the powder have a median diameter less than about 50 μm, for example, between about 1.5 μm and 10 μm. The mean particle diameter can be measured using conventional equipment, such as a Cascade Impactor (Andersen, Ga.).

[0128] The powder may be suspended in a propellant with the aid of a surfactant. The propellant may be one of a variety of conventional materials employed for this purpose, such as a chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol, and 1,1,1,2-tetrafluoroethane, or combinations thereof. Suitable surfactants include sorbitan trioleate and soya lecithin. Oleic acid may also be useful as a surfactant.

[0129] In certain embodiments of the invention, the pharmaceutical compositions are administered intranasally and the compositions are therefore formulated as nasal gels, creams, pastes or ointments that provide a more sustained contact with the nasal mucosal surfaces. These formulations typically have a viscosity between about 10 and about 250, 000 centipoise (cps), for example, between about 2500 about 100,000 cps, or between about 5,000 and 50,000 cps. Such formulations may be based upon, for example, alkylcelluloses and/or other biocompatible carriers of high viscosity well known to the art. A non-limiting example of an alkylcellulose is methylcellulose, which can be included in a suitable concentration, for example, between about 5 mg and about 1000 mg per 100 ml of carrier, or between about 25 mg and about mg per 100 ml of carrier. In certain embodiments, the carrier containing the PapMV moiety may be soaked into a suitable substrate, for example a fabric material, such as gauze, that can be applied to the nasal mucosal surfaces to allow for penetration of the PapMV moiety into the mucosa.

[0130] In certain embodiments, gel formulations may also include a permeation enhancer (penetration enhancer). Permeation enhancers include, but are not limited to, sulfoxides such as dim ethylsulfoxide and decylmethylsulfoxide; surfactants such as sodium laurate, sodium lauryl sulfate, cetyltrimethylammonium bromide, benzalkonium chloride, poloxamer (231, 182, 184), Tween (20, 40, 60, 80) and lecithin; the 1-substituted azacycloheptan-2-oncs, particularly 1-n-dodecylcyclazacycloheptan-2-one; fatty alcohols such as lauryl alcohol, myristyl alcohol, oleyl alcohol and the like; fatty acids such as lauric acid, oleic acid and valeric acid; fatty acid esters such as isopropyl myristate, isopropyl palmitate, methylpropionate, and ethyl oleate; polyols and esters thereof such as propylene glycol, ethylene glycol, glycerol, butanediol, polyethylene glycol, and polyethylene glycol monolaurate, amides and other nitrogenous compounds such as urea, dim-

ethylacetamide (DMA), dimethylformamide (DMF), 2-pyrrolidone, 1-methyl-2-pyrrolidone, ethanolamine, diethanolamine and triethanolamine, terpenes; alkanones, and organic acids, particularly salicylic acid and salicylates, citric acid and succinic acid. The permeation enhancer may be present in an amount from about 0.1% to about 30% w/w. The gel compositions may also include a buffering agent, for example, carbonate buffers, citrate buffers, phosphate buffers, acetate buffers, hydrochloric acid, lactic acid, tartaric acid, inorganic and organic bases. The buffering agent may be present in a concentration of about 1 to about 10 weight percent, for example, about 2 to about 5 weight percent, depending on the type of buffering agent(s) used, as known by the one skilled in the art. Concentrations of the buffering agent(s) may vary, however, and in some embodiments the buffering agent may replace up to 100% of the water amount within the composition.

[0131] In certain embodiments of the invention, the pharmaceutical compositions are formulated for rectal or vaginal administration and may be presented as a suppository, which may be prepared by mixing the active ingredient(s) with one or more suitable non-irritating excipients or carriers. Non-limiting examples of excipients or carriers include cocoa butter, polyethylene glycol, a suppository wax or salicylate and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active ingredient(s). Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

[0132] Also encompassed by the present invention are pharmaceutical compositions comprising the PapMV moiety in combination with commercially available vaccines, for example, compositions formulated for intranasal or pulmonary administration.

[0133] Other pharmaceutical compositions and methods of preparing pharmaceutical compositions are known in the art and are described, for example, in "*Remington: The Science and Practice of Pharmacy*" (formerly "*Remingtons Pharmaceutical Sciences*"); Gennaro, A., Lippincott, Williams & Wilkins, Philadelphia, Pa. (2000).

Methods and Uses

[0134] The present invention provides for the use of PapMV compositions to stimulate the innate immune response in a subject. The subject may be a human or a non-human animal. The compositions are useful, for example, in the treatment or prevention of infection, including chronic infection.

[0135] Certain embodiments provide for administration of PapMV compositions to a subject to protect the subject from potential infection by a pathogen. In accordance with certain embodiments of the invention, the PapMV compositions are administered to elicit a protective effect within the mucosa and/or in the respiratory system. Administration via intranasal or pulmonary routes, for example, can be used to provide protection against respiratory pathogens. Administration via vaginal routes, for example, can be used to provide protection against vaginal pathogens. Intranasal administration may also be effective to provide protection against vaginal pathogens (see, for example, Holmgren & Czerkinsky, 2005, *Nature Medicine*, 11(4):S45-S53). Other routes of administration are also contemplated.

[0136] The innate immune response stimulated by administration of PapMV compositions is non-specific and thus is expected to provide protection against or treatment of a broad range of pathogens. The effects are rapid and thus may find utility in situations that require more immediate protection than a traditional vaccine may provide. In addition, as the effects are non-specific, administration of PapMV compositions could provide immediate protection while the identity of the pathogen is determined and an appropriate vaccine or other treatment identified.

[0137] Certain embodiments of the invention provide for the administration of PapMV compositions to a subject as a preventative or pre-emptive measure to protect against infection with a pathogen. Such an approach is useful, for example, in immunocompromised patients (such as patients with AIDS, patients under chemotherapy or patients taking immunosuppressive drugs), in pandemic or epidemic situations to provide initial protection to the population prior to development/distribution of an appropriate vaccine, to protect workers such as rescue workers, doctors and nurses entering areas of potential infection, and in situations where there is a threat of, or an incidence of, a bioterrorism attack.

[0138] As demonstrated herein, administration of PapMV compositions increased production of cytokines and chemokines within about 6 hours or less of administration and protection against challenge lasted for several days. Accordingly, in certain embodiments, the present invention contemplates the prophylactic administration of PapMV compositions to a subject between about 4 hours and about a week prior to predicted exposure to a suspected pathogen, for example, at least 4, 5, 6, 7, 8, 9, 10, 11 or 12 hours prior to exposure and up to about 7, 6, 5 or 4 days prior to exposure. Various ranges between each of these upper and lower limits are also contemplated in certain embodiments. For example, between about 4, 5, 6, 7, 8, 9, 10, 11 or 12 hours and about 7 days, between about 4, 5, 6, 7, 8, 9, 10, 11 or 12 hours and about 6 days, between 4, 5, 6, 7, 8, 9, 10, 11 or 12 hours and about 5 days, between about 4, 5, 6, 7, 8, 9, 10, 11 or 12 hours and about 4 days.

[0139] Certain embodiments provide for the administration of multiple doses of the PapMV compositions in order to prolong the protection period. Administration of the doses would take place at specified time intervals, for example, doses could be spaced by a period of between about 12 hours to about 10 days. Certain embodiments provide for the administration of a first dose followed by at least one subsequent dose and up to about 10 subsequent doses at time intervals of at least about 12, 24, 36, 48 or 72 hours and up to about 10, 9, 8 or 7 days, for example, between about 24 hours and about 7 days.

[0140] In certain embodiments, PapMV compositions may be administered to provide protection against potential infection with a bacterial pathogen. Bacterial pathogens include, for example, various species of the *Bacillus, Yersinia, Franscisella, Haemophilus, Streptococcus, Staphylococcus, Pseudomonas, Mycobacterium*, and *Burkholderia* genus of bacteria. In certain embodiments, PapMV compositions may be administered to provide protection against potential respiratory infection with a bacterial pathogen. Non-limiting examples of relevant pathogenic bacterial species include, but are not limited to, *Bacillus anthracis, Yersinia pestis, Francisella tularensis, Streptococcus pnemoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Burkholderia cepacia, Corynebacterium diphtheriae, Legionella pneumophila,*

Mycoplasma pneumoniae, Chlamydophila pneumoniae, Mycobacterium tuberculosis, Moraxella catarrhalis, Haemophilus influenzae, Klebsiella pneumoniae, Escherichia coli, Coxiella burnetii, Clostridia spp. and *Shigella* spp. In certain embodiments of the invention, PapMV compositions may be administered to provide protection against potential infection with a bacteria associated with bacterial pneumonia, for example, one or more of *S. pneumoniae, S. aureus, H. influenzae, K. pneumoniae, P. aeruginosa, E. coli, M. catarrhalis, C. burnetii, M. pneumoniae, L. pneumoniae, C. pneumoniae* and *Y. pestis*. In certain embodiments of the invention, PapMV compositions may be administered to provide protection against infection with a vaginal or intestinal bacterial pathogen, for example, *Shigella* spp., *Salmonella* spp., *E. coli* or *Chlamydia trachomatis*.

[0141] In certain embodiments, PapMV compositions may be administered to provide protection against potential infection with a viral pathogen. Viral pathogens include, for example, viruses from the family Adenoviradae; Arenaviridae (for example, Ippy virus and Lassa virus); Birnaviridae; Bunyaviridae; Caliciviridae; Coronaviridae; Filoviridae; Flaviviridae (for example, yellow fever virus, dengue fever virus and hepatitis C virus); Hepadnaviradae (for example, hepatitis B virus); Herpesviradae (for example, human herpes simplex virus 1); Orthomyxoviridae (for example, influenza virus A, B and C); Paramyxoviridae (for example, mumps virus, measles virus and respiratory syncytial virus); Picornaviridae (for example, poliovirus and hepatitis A virus); Poxyiridae; Reoviridae; Retroviradae (for example, BLV-HTLV retrovirus, HIV-1, HIV-2, bovine immunodeficiency virus and feline immunodeficiency virus); Rhabdoviridae (for example, rabies virus), and Togaviridae (for example, rubella virus). Non-limiting examples of relevant pathogenic viruses include, but are not limited to, various strains of the influenza virus, cytomegalovirus, various strains of respiratory syncytial virus (including human respiratory syncytial virus and specific animal strains), various strains of parainfluenza virus (including human parainfluenza virus and specific animal strains), coronavirus (including human coronavirus and SARS coronavirus), rhinovirus (including human rhinovirus), enterovirus (including human enterovirus), adenovirus (including human adenovirus), bocavirus (including human bocavirus), metapneumovirus (including human metapneumovirus), dengue virus, various hepatitis viruses, human immunodeficiency virus (HIV), West Nile virus, rabies virus, human papilloma virus (HPV), Epstein Barr virus (EBV) and polyoma virus. In certain embodiments of the invention, PapMV compositions may be administered to provide protection against potential infection with an influenza virus, a flavivirus (such as dengue fever virus or yellow fever virus), a parainfluenza virus, human metapneumovirus, respiratory syncytial virus, coronavirus (such as SARS coronavirus), a rhinovirus or an adenovirus.

[0142] In certain embodiments, PapMV compositions may be administered to provide protection against potential infection with a fungal pathogen. Fungal pathogens include, for example, *Histoplasma capsulatum, Coccidiodes immitis, Blastomyces dermatitidis, Cryptococcus neoformans, Aspergillus fumigatus, Candida albicans* and *Pneumocystis carinii*.

[0143] In certain embodiments, PapMV compositions may be administered to provide protection against a biological weapon/bioterrorism agent. Examples of bioterrorism agents include, Category A bioterrorism agents such as anthrax (*Bacillus anthracia*), botulism (*Clostridium botulinum* toxin), plague (*Yersinia pestis*), smallpox (variola major), tularemia (*Francisella tularensis*) and viral hemorrhagic fevers (filoviruses, such as Ebola and Marburg, and arenaviruses, such as Lassa and Machupo); Category B bioterrorism agents such as brucellosis (*Brucella* species), epsilon toxin of *Clostridium perfringens*, glanders (*Burkholderia mallei*), melioidosis (*Burkholderia pseudomallei*), psittacosis (*Chlamydia psittaci*), Q fever (*Coxiella burnetii*), ricin toxin from *Ricinus communis* (castor beans), staphylococcal enterotoxin B, typhus fever (*Rickettsia prowazekii*) and viral encephalitis (alphaviruses, such as Venezuelan equine encephalitis, eastern equine encephalitis and western equine encephalitis); and Category C bioterrorism agents that include emerging infectious diseases such as Nipah virus and hantavirus.

[0144] In certain embodiments, PapMV compositions may be administered to animals in competition settings as a preemptive measure to protect against infection, for example, horse races, dog shows, cat shows and the like. Administration of PapMV compositions to livestock in epidemic/pandemic situations is also contemplated in certain embodiments. Examples of common animal pathogens include, but are not limited to, *Bordetella bronchiseptica* (the most common causative agent of "kennel cough"), canine distemper virus, canine adenovirus (type 1 or 2), canine parainfluenza-virus (CPI), canine influenza virus (CIV), canine reovirus (type 1, 2 or 3), canine herpes virus, feline herpesvirus, feline calicivirus (FCV), *Chlamydophila [Chlamydia] psittaci*, bovine respiratory syncytial virus (BRSV), Bovine Viral Diarrhea (BVD) virus, Parainfluenza Type 3 (P13) virus, *Haemophilus somnus, Mannheimia* (*Pasteurella*) *haemolytica, Pasteurella multocida*, African swine fever virus (ASFV), classical swine fever virus (CSFV), peste de petits ruminants virus (PPRV), Nairobi sheep disease virus (NSDV), *Actinobacillus pleuronomiae, Mycoplasma hyopneumoniae*, swine influenza virus, various strains of avian influenza virus, equine arteritis virus, equine herpesvirus, various strains of equine influenza virus, *Rhodococcus equi* and *Streptococcus* equi (for example, subspecies *equi* and *zooepidemicus*).

[0145] In certain embodiments, PapMV compositions may be used in combination with intra-nasal vaccines to augment and extend the effects of these vaccines. For example, certain influenza vaccines have been developed for instranasal administration. Vaccines against pneumococcal bacteria and *Yersinia pestis*.

[0146] In certain embodiments, PapMV compositions are administered to stimulate the mucosal immune response in general and thus improve protection to diseases or infections of the intestine, genitourinary tract, and other mucosal surfaces including the lung. The PapMV compositions can be administered, for example, via intranasal or pulmonary routes or intravaginally. In certain embodiments, PapMV compositions are administered in combination with one or more antigens to stimulate the mucosal immune response. In this context, the PapMV compositions are acting as a mucosal adjuvant that augments the effects of the antigen(s) in order to generate an effective mucosal immune response. Certain embodiments of the invention, therefore, provide for the use of the PapMV compositions, alone or in combination with one or more antigen, to provide protection against infection with a micro-organisms that gain access to the body via mucosal membranes. Examples of such micro-organisms include, but are not limited to, *Helicobacter pylori, Vibrio*

cholerae, Escherchia coli, Shigella spp., *Clostridium difficile*, rotaviruses, calici viruses, *Mycoplasma pneumoniae*, influenza virus, *Mycobacterium tuberculosis*, respiratory syncytial virus, HIV, *Chlamydia trachomatis, Neisseria gonorrhoeae* and herpes simplex virus.

[0147] In certain embodiments, PapMV compositions may be used to treat an infection, for example, an infection with a viral pathogen, a bacterial pathogen or a fungal pathogen such as those described above. In some embodiments, PapMV compositions may be used to treat an infection at a mucosal surface, for example, in the lungs, intestines or genitourinary system.

[0148] Some embodiments of the invention provide for the use of PapMV compositions to decrease the viral load in a subject with a chronic, persistent or recurrent infection and thus assist with management and/or clearance of the infection. Examples of chronic infections include, but are not limited to, HIV, AIDS and hepatitis C virus (HCV) infections. Examples of persistent and/or recurrent infections include, but are not limited to, hepatitis B virus (HBV) infections, herpes simplex virus (HSV) infections, tuberculosis (caused by *Mycobacterium tuberculosis* infection) and lyme disease (caused by *Borrelia burgdorferi* infection).

[0149] Combination therapies using PapMV compositions and conventional therapies for chronic infection are also provided in some embodiments. For example, in certain embodiments, PapMV compositions may be used in combination with PEG-interferon, ribavirin or PEG-interferon/ribavirin treatment for HCV, or in combination with nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs), highly active anti-retroviral therapy (HAART), fusion inhibitors or non-nucleoside reverse transcriptase inhibitors (NNRTT) for HIV and AIDS. In some embodiments, such combination therapies may, for example, result in one or more of an improved efficacy of the conventional therapy, a decrease in the dosage amount of the conventional therapy required to reach a predetermined endpoint, a decrease in the duration of treatment, a decrease in side-effects associated with the conventional therapy, or the like.

[0150] Certain embodiments of the invention provide for the use of PapMV compositions, alone or in combination with a conventional therapy, to treat immune exhaustion in a subject with a chronic, persistent or recurrent infection.

[0151] In certain embodiments, PapMV compositions can be administered via pulmonary routes to lung cancer patients to stimulate the anti-tumour activity of the innate immune response in the lungs.

Kits

[0152] The present invention additionally provides for kits comprising PapMV compositions. In certain embodiments the kit is portable and may be carried on a person. The kit may optionally further include a pathogen detector. The kit may also optionally contain a gas or mechanical propellant for the PapMV compositions.

[0153] Individual components of the kit would be packaged in separate containers and, associated with such containers, can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale. The kit may optionally contain instructions or directions outlining the method of use or administration regimen for the PapMV composition.

[0154] The components of the kits may be packaged as solutions, pessaries or in powdered or lyophilized form. When components of the kit are provided in dried or lyophilised form, the kit can additionally contain a suitable solvent for reconstitution of the dried or lyophilised components. Irrespective of the number or type of containers, the kits of the invention also may comprise an instrument for assisting with the administration of the composition to a patient. Such an instrument may be an inhaler, nebulizer, nasal spray device, syringe, pipette, pessary dispenser or similar medically approved delivery vehicle. In certain embodiments, the container comprising the composition may itself be such an instrument.

[0155] To gain a better understanding of the invention described herein, the following examples are set forth. It will be understood that these examples are intended to describe illustrative embodiments of the invention and are not intended to limit the scope of the invention in any way.

EXAMPLES

Example 1

Induction of an Antiviral Response in Mice by Administration of PapMV VLPs #1

[0156] PapMV VLPs were prepared by expression of the PapMV coat protein and self-assembly in *E. coli* cells as previously described (Tremblay et al., 2006, *FEBS J.*, 273: 14-25) using PapMV coat proteins having a sequence as set forth in SEQ ID NO:4 (FIG. **2**B). Balb/C mice (10 per group) were treated twice at 7-day intervals with either 30 or 75 μg (50 μL volume) of PapMV VLPs administered by the intranasal route followed by an intranasal challenge with 100 pfu (plaque forming unit) of influenza virus strain WSN/33 three days after the second treatment. The development of infection was followed for 14 days. The weight of the animal was measured once per day during these 14 days. Animals that showed more that 20% weight loss were sacrificed. The control group was treated with PBS (saline buffer).

[0157] The results are shown in FIG. **3**A-C. Both groups treated with the PapMV VLPs did not show signs of infection and continued to gain weight normally (FIGS. **3**A & B). In contrast, the group treated with PBS showed signs of infection and had lost more than 12% of their weight at day 8 after challenge (FIGS. **3**A & B). PapMV VLPs improved the capacity of the animals to fight influenza infection. All mice treated with the PapMV VLPs survived to the end of the experiment, whereas 30% of the control group did not survive beyond day 8 after challenge (FIG. **3**C).

Example 2

Duration of Protection to Viral Challenge Induced by PapMV VLPs

[0158] A similar experiment to that described in Example 1 was performed. The PapMV VLPs were prepared by the same method and the same schedule of treatments was employed, but using a different dose of PapMV VLPs (60 μg). The challenge with influenza virus strain WSN/33 (200 pfu) was performed at days 10, 12, 14 and 17 (i.e. 3, 5, 7 and 10 days after PapMV treatment) and symptoms were monitored for 14 days after challenge.

[0159] The results are shown in FIGS. **4**A-C and show that PapMV VLPs provided protection for up to 5 days after the

last treatment. Protection was evidenced by decreased weight losses (FIG. **4**A) and lesser symptoms (FIG. **4**B) compared to control animals, as well as 100% survival (FIG. **4**C), in those animals challenged either 3 or 5 days after the last treatment with PapMV VLPs. The induced protection however faded by day 7, with a less than 50% survival rate observed in mice challenged at 7 or 10 days after the last treatment with PapMV VLPs, a survival rate comparable with the control group treated with PBS.

[0160] The protection induced by the treatment with PapMV VLPs thus appears to persist for a period of about 5 days, a period that is consistent with a non-specific and non-persistent induction of the innate immune response.

Example 3

Induction of an Antiviral Response in Mice by Administration Of PapMV VLPs Containing Synthetic ssRNA

[0161] Polyinosinic-polycytidylic acid (poly I:C; dsRNA), a well known Toll-like receptor 3 (TLR-3) ligand, has been shown to be an inducer of the innate immune response in lungs through induction of the secretion of pro-inflammatory cytokines such as IL-6, CXCL10, JE, KC, mGCSF, CCL3, CCL5, and TNF (Stowell et al., 2009, *Respir. Res.*, 10:43). TLR-7 is also known to activate the innate immune response through the binding of ligands such as ssRNA and R837 (a guanosine analogue).

[0162] In an attempt to increase the capacity of the PapMV VLPs to elicit an innate immune response and the development of an antiviral response, PapMV VLPs containing either poly I:C dsRNA or ssRNA were prepared by the method described in Example 17. PapMV coat protein was assembled in vitro with either poly I:C (dsRNA; InvivoGen, San Diego, Calif.) or ssRNA to produce VLPs comprising the respective RNAs. The ssRNA was prepared in vitro using the Promega T7 Ribomax Express large scale RNA production system (Promega, Madison, Wis.).

[0163] The assembled VLPs were examined by electron microscopy and observed to be similar to VLPs prepared by the method described in Tremblay et al. (2006, *FEBS* 1, 273:14-25) (see FIG. **25**A: PapMV VLPs containing ssRNA and FIG. **25**B: PapMV VLPs containing poly I:C).

[0164] The efficacy of the two types of VLPs in inducing protection against challenge with influenza virus was evaluated. Balb/C mice (10 per group) were treated with 60 μg of PapMV VLPs containing ssRNA ("PapMV VLP ssRNA"), PapMV VLPs containing poly I:C ("PapMV VLP poly I:C") or with an equivalent amount of RNA (i.e. 3 μg of either poly I:C or ssRNA). Control mice were treated with 60 μg of PapMV coat protein (CP) monomers (without RNA) or with control buffer (10 mM Tris-HCl pH 8). Mice were treated as described in Example 2 (i.e. intranasally twice at 7 day intervals) and challenged 3 days after the last treatment with 200 pfu of influenza virus strain WSN/33. The weight, symptoms and survival of the animals were measured once per day during the following 14 days. Animals that showed more that 20% weight loss were sacrificed.

[0165] The results are shown in FIG. **5**A-B. Mice treated with PapMV VLP ssRNA showed the best performance of the treated groups. Specifically, mice treated with PapMV VLP ssRNA did not lose any significant amount of weight (FIG. **5**A) and showed very few, if any, symptoms (FIG. **5**B). The groups treated with either PapMV VLP poly I:C or poly I:C alone showed partial protection to the challenge with decreased weight losses (FIG. **5**A) and symptoms (FIG. **5**B) as compared to the control group. Treatment with the PapMV CP monomers did not provide any protection with the amount of weight loss (FIG. **5**A) and symptoms (FIG. **5**B) observed in mice treated with the monomers being similar to that observed in mice treated with the PBS control. Subsequent analysis of the PapMV VLP poly I:C suggested that these VLPs are not as stable as the PapMV VLP ssRNA, which may account for their poorer performance.

Example 4

Induction of Cytokines in Mice by Administration of PapMV VLPs #1

[0166] To elucidate the mechanisms induced by the PapMV VLP in the lungs, mice (5 per group) were treated following the same protocol as described in Examples 1 to 3 above (i.e. 2 treatments intranasally at 7 day intervals) with 60 μg PapMV VLPs containing ssRNA, 15 μg of PamCSK4 (a TLR-2 ligand and non-inducer of IFN type 1) (Cedarlane, Burlington, ON) or with the control buffer (10 mM Tris HCl pH8). Broncho-alveolar lavage (BAL) was performed 24 hours after the second treatment and screened for the presence of cytokines using Luminex technology (Milliplex Mouse cytokine premixed 32-plex immunoassay kit; Millipore).

[0167] Two major cytokines, interleukin-9 (IL-9) and interferon-γ-induced protein 10 kDa (IP-10), were induced by treatment with PapMV VLPs or PamCSK4 (FIGS. **6**A & B). IL-9 is a cytokine secreted by CD4+ T lymphocytes that promotes T-cell proliferation and inhibition of apoptosis. IP-10 appears as a result of the secretion of IFN-γ and plays an important role in recruitment of T-lymphocytes, dendritic cells, NK cells and macrophages at the site of stimulation. The induction of both cytokines by PamCSK4 (which is a known a TLR-2 ligand and pathogen associated molecular patterns (DAMP) molecule) and PapMV VLPs suggests that the VLPs may also be PAMPs.

Example 5

Induction Of Cytokines in Mice by Administration of PapMV VLPs #2

[0168] A similar experiment to that described in Example 4 was conducted except that the BAL was performed 6 hours after treatment, and the treatments were either 1 or 2 administrations at 7 day intervals. As before, 60 μg of PapMV VLPs containing ssRNA were used in the experiment. Luminex (32 cytokines detection kit) was used to screen for cytokine production early after treatment.

[0169] The results are shown in FIGS. **7**A-R and demonstrate that 2 treatments with PapMV VLPs were more efficient than one treatment in inducing cytokines and chemokines in mice. In addition, a wider variety of cytokines and chemokines were detected at 6 hours after treatment than 24 hours after treatment (compare FIGS. **6** and **7**).

[0170] MIP-1α, MIP-1β, MIP-2, mKC, TNF-α and MCP-1 were found to be very abundant (FIGS. **7**A-E and H) in BAL from mice treated with PapMV VLPs. These cytokines and chemokines activate human granulocytes (neutrophils, eosinophils and basophils) which can lead to acute neutrophilic inflammation. They also induce the synthesis and release of other pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1α/β from fibroblasts and macrophages (Maurer and von

Stebut, 2004, *The International Journal of Biochemistry & Cell Biology.* 36: 1882-1886), which were also shown to be induced by PapMV VLPs (see FIGS. **7**E, N, O and P respectively). MIP-1 proteins can also promote health by inducing inflammatory responses against infectious pathogens such as viruses, including influenza virus (Menten et al., 2002, *Cytokine Growth Factor Reviews,* 13: 455-481) and parasites (Aliberti et al., 2000, *Nature Immunology,* 1: 83-87), which is consistent with the results shown in the preceding Examples.

[0171] IL-6 was also observed to be secreted in response to administration of PapMV VLPs (FIG. **7**N). Interestingly, IL-6 secretion was showed to be required for resistance to infection by the bacteria *Pneumococcus pneumoniae* (van der Poll et al., 1997, *J. Infect Dis.,* 176 (2): 439-44).

[0172] IP-10 was strongly induced by the treatment with PapMV VLPs (FIG. **7**I). IP-10 is a chemotactic chemokine that favours the recruitment of T cells at inflammatory sites and also favours proliferation and activation of natural killer cells (NK cells).

[0173] Interleukin 17 was also induced by the treatment with PapMV VLPs (FIG. **7**J). IL-17 is a cytokine that acts by increasing chemokine production in various tissues to recruit monocytes and neutrophils to the site of inflammation, similar to Interferon gamma. IL-17 is produced by T helper cells and is also a proinflammatory cytokinc that responds to the invasion of the immune system by extracellular pathogens. IL-17 coordinates local tissue inflammation through the upregulation of proinflammatory cytokines and chemokines such as IL-6. granulocyte colony-stimulating factor, TNFα, IL-1, KC, MCP-1 and MIP-2 (Zepp et al., 2011, *Trends Immunol. April* 12. [Epub ahead of print]), which were also shown to be induced by PapMV VLP treatment.

[0174] PapMV VLP treatment (FIGS. **7**Q & R) also induced G-CSF and GM-CSF, which are known to stimulate stem cells to produce granulocytes (neutrophils, eosinophils and basophils) and monocytes. Monocytes exit the circulation and migrate into tissue, whereupon they mature into macrophages. Thus, G-CSF and GM-CSF are part of the inflammatory cascade by which activation of a small number of macrophages can rapidly lead to an increase in their numbers, a process crucial for fighting infection (Metcalf, 2010, *Nature Reviews Cancer,* 20: 425-434).

[0175] The results described in Examples 4 and 5 demonstrate that the treatment of mice with PapMV VLPs induces a strong and general inflammatory response as showed by the profile of cytokines and chemokines that are secreted by the immune cells. The levels of cytokines and chemokines were maximal at 6 hours after treatment and decreased significantly 24 hours after treatment. It is likely that the inflammatory cytokines and chemokines induced the migration of immune cells and granulocytes and thus are responsible for the anti-viral state of the animal for more than 5 days. The induced cytokines can also lead to secretion of IFN type 1 that in turn is also known to provide an anti-influenza activity.

Example 6

Activation of TLR-7 by PapMV VLP ssRNA

[0176] C57BL/6, TLR7 knockout (KO), MYD88 KO and IRF5/7 KO mice (3-5 mice per group) were immunized intravenously (i.v.) with 100 μg PapMV VLP ssRNA or 100 μl PBS. Splenocytes were isolated 24 hours post-immunization and CD86 and CD69 expression in dendritic cells (DCs), $CD8^+$ T cells and B cells was analyzed. Cells were sorted by FACS and the level of CD86 and CD69 was evaluated by fluorescence intensity though the binding of a CD69 or CD86 specific antibody. The results are presented in FIG. **8** as a ratio of the Mean Fluorescence Intensity (MFI) of the analyzed sample on the PBS sample.

[0177] In brief, these results show that antigen-presenting cells, such as DCs and B cells and CD8+ T cells, are activated by PapMV VLP ssRNA nanoparticles. Activation is dependent on IRF5/7, Myd88 and TLR-7, as activation is lost in mice that are knockouts in IRF5/7, Myd88 or TLR7. It is believed that TLR-7 is triggered through the ssRNA that is contained in the VLPs. Experiments performed with the coat protein of PapMV (in monomeric or other low molecular weight form) failed to activate TLR-7.

[0178] IRF5/7 are the interferon responsive factors that are induced upon stimulation of TLR-7 and lead to production of interferon alpha. The Myd88 molecule is an adaptor molecule that is responsible for the transfer of the signals triggered by TLR-7. The cascade of the reaction is proposed to be: 1) triggering of TLR-7 by the ssRNA in the VLPs, and 2) engagement of Myd88 followed by the induction of IRF5/7 that will lead to an increase in interferon alpha production. Finally, interferon alpha will contribute to the immunomodulation effects of the PapMV VLP nanoparticles.

Example 7

Involvement of Plasmacytoid Dendritic Cells in PapMV VLP Immunogenicity

[0179] C57BL/6 mice (5 per group) were immunized i.v. with 100 μg PapMV VLP ssRNA either with or without prior treatment to deplete BST2+ cells. For depletion, C57BL/6 mice were injected i.p. with 500 μg of an anti-BST2 antibody (mAb 927) at 4811 and 24 h prior to PapMV VLP ssRNA immunization. CD69, MHC—I and CD86 expression in isolated splenocytes was analyzed by FACS at 24 h after PapMV VLP ssRNA immunization.

[0180] The results are shown in FIG. **9** and indicate that $BST2^+$ cells (mainly plasmacytoid dendritic cells) are important for the immunogenicity of PapMV VLP ssRNA nanoparticles in mice. Specifically, it was observed that in mice in which BST2+ cells were depleted, activation of B cells, CD8+ cells and DCs was lost, suggesting that the activation is going through the plasmacytoid dendritic cells.

Example 8

Stimulation of Interferon-α Production of by PapMV VLPs #1

[0181] Two groups of C57BL/6 mice, as well as TLR-7 KO and MYD88 KO mice (4 mice per group) were immunized i.v. with 100 μg PapMV VLP ssRNA or 100 μl PBS. One group of C57BL/6 mice had first been treated with anti-BST2 antibody as described in Example 7. IFN-α production in serum and spleen was monitored by ELISA (VeriKine™ Mouse Interferon Alpha ELISA Kit; PBL InterferonSource) at either 6, 12, 24 and 48 h post-immunization (FIG. **10**A) or at 6 h after the immunization (FIG. **10**B).

[0182] The results are shown in FIG. **10** and indicate that IFN-α production stimulated by PapMV VLP ssRNA nanoparticles depends on MYD88, ILR7 and $BST2^+$ cells.

Example 9

Stimulation of Interferon-α Production of by PapMV VLPs #2

[0183] C57BL/6 and IFNAR KO mice (3 mice per group) were immunized i.v. with 100 μg PapMV VLP ssRNA or 100 μl PBS. CD86, MHC-I and CD69 expression in B lymphocytes and dendritic cells isolated from the spleens of the mice 24 h after immunization was assessed by flow cytometry.

[0184] The results are shown in FIGS. **11**A and B, and indicate that the type I IFN receptor is necessary for the activation of murine immune cells by PapMV VLP ssRNA nanoparticles. Mice that were knockouts for the type I IFN receptor (IFNAR KO) did not show activation of the immune cells by PapMV VLP ssRNA nanoparticles.

[0185] Levels of antibody against PapMV VLP ssRNA in the serum of C57BL/6 and IFNAR KO mice (9 mice per group) at day 4, 8, 12, 20 and 30 after immunization with 100 μg PapMV VLP ssRNA were analyzed by indirect ELISA measuring total IgG binding to PapMV VLP ssRNA coated plate.

[0186] The results are shown in FIG. **11**C and indicate that the absence of type I IFN signalling causes a significant delay in the antibody response against the PapMV VLP ssRNA nanoparticles.

Example 10

Pre-Treatment with PapMV VLPs Helps to Control Chronic Infection

[0187] LCMV is a relevant animal model of chronic infection (such as HCV infection). The clone 13 variant of LCMV establishes a persistent infection in mice. LCMV infection, like HCV infection, is largely controlled by CTLs and exhaustion of the CTL response is associated with PD-1 expression.

[0188] C57BL/6 and TLR7 knockout (KO) mice (3-6 mice per group) were treated i.v. with 100 μg PapMV VLP ssRNA, 100 μg R837 (a commercially available TLR-7 ligand) or 100 μl PBS 6 hours before infection (i.v.) with 2×10^6 PFU LCMV clone 13. Blood samples were taken at day 5, 11, 15, 25 and 45 to evaluate the viral titer by LCMV focus-forming assay. Mice were sacrificed 15 days or 45 days post-infection for analysis of the immune response in the spleen by FACS and of the viral titer in the spleen, liver, kidney and brain by LCMV focus-forming assay on MC57 fibroblasts using a rat anti-LCMV-NP monoclonal Ab (VL-4) as previously described (Lacasse et al., 2008, *J. Virology,* 82:785-794). The viral kinetics of LCMV clone 13 in the blood of the C57BL/6 mice are depicted in FIG. **12** and show that pre-treatment with PapMV VLP ssRNA nanoparticles control chronic infection induced by LCMV.

[0189] The viral titers in spleen, kidney, liver and brain of C57BL/6 and TLR7KO mice at day 15 post-infection are shown in FIG. **13** and demonstrate that pre-treatment with PapMV VLP ssRNA nanoparticles decreases the viral load in different organs with greater efficiency than a commercial TLR7 ligand (R837) and in a TLR7 dependent manner. It is believed that the TLR-7 ligand in the PapMV VLP ssRNA nanoparticics is the ssRNA component, which represents approximately 5% of the molecule. As such, although 100 μg of each was administered to the mice, the PapMV VLP ssRNA nanoparticles are more than 20-fold more effective than R837 in reducing the LCMV viral load in the mice.

[0190] FIG. **14** shows that administration of PapMV VLP ssRNA nanoparticles before infection with LCMV clone 13 increases the functionality of GP33 specific $CD8^+$ T cells. Similar results were obtained for NP396 specific $CD8^+$ T cells. In particular, FIG. **14**F shows that the amount of PD-1 expressed in GP33 specific $CD8^+$ T lymphocytes is significantly decreased by pre-treatment with PapMV VLP ssRNA nanoparticles. PD-1 is an indicator of immune exhaustion and its expression is a characteristic of LCMV clone 13 infection. Pre-treatment of the mice with PapMV VLP ssRNA nanoparticles resulted in the PD-1 level remaining as low as in the uninfected mice suggesting that the immune system is not exhausted in these mice, which is why they are able to resist infection.

[0191] FIG. **15** shows the viral titers in spleen, kidney, liver and brain of C57BL/6 mice at day 45 post-infection. This result indicates that the decrease in viral load resulting from pre-treatment with PapMV VLP ssRNA nanoparticles is still evident several weeks after treatment.

Example 11

Activation of Human Monocytes In Vitro by PapMV VLPs

[0192] Human PBMCs were isolated by Ficoll gradient and treated with 100 μg/ml PapMV VLP ssRNA or PBS. At 18 h post-treatment, $CD14^+CD11b^+$ cell population (monocytes) were analyzed for CD86 expression by flow cytometry.

[0193] The results are shown in FIG. **16** and indicate that human monocytes are also activated by PapMV VLP ssRNA nanoparticles. These results are representative of three independent experiments.

Example 12

Induction of an Anti-Bacterial Response by PapMV VLPs

[0194] Mice, 10 per group, were treated twice at 7-day intervals via the intranasal route with buffer alone (10 mM Tris pH8) or with 60 μg of PapMV VLP ssRNA. At day 3 post-treatment, the mice were infected with 220 CFU (colony forming units) of a virulent *Streptococcus pneumoniae* strain.

[0195] Survival was monitored closely every 12 hours over 4 days. The results are shown in FIG. **17**. All mice in the group treated with PapMV VLP ssRNA nanoparticles survived the infection. The group treated with the buffer showed 70% survival.

[0196] Although the dose of *Streptococcus pneumoniae* used in this Example was a sub-lethal dose, the data strongly suggests that pre-treatment with PapMV nanoparticles will provide protection against a bacterial infection through the induction of an innate immune response in the lungs. This example and the preceding examples demonstrate that the protection conferred by the PapMV nanoparticles is non-specific as it is effective against infection with viruses and bacteria.

Example 13

Treatment of LCMV Chronic Infection Using PapMV VLPs

[0197] C57BL/6 Mice (3 per group) were infected i.v. at day 0 with 2×10^6 PFU LCMV clone 13 and treated i.v. once/

day with 100 μg PapMV VLP ssRNA or 100 μl PBS either at days 1, 2, 3, 4 and 5 (Group A), or at days 6 and 7 only (Group B). Blood samples were taken at day 5, 10 and 15 and mice were sacrificed at day 15 post-infection for analysis of the viral titer by LCMV focus-forming assay in blood, spleen, kidney and brain.

[0198] Viral titers found in the blood of the animals are shown in FIG. **18**. Although at day 15, mice treated with PapMV VLP ssRNA nanoparticles showed the same titers as the controls, a significant reduction of viral titers was observed at day 10 in both groups of mice (close to a log 10 reduction in the animals of Group A). This result strongly suggests that, with adjustment to the treatment regimen, further decreases in viral load in mice treated with PapMV VLP ssRNA nanoparticles will be achievable. For example, the number of treatments could be increased. As treatments provided at days 1 to 5 or 6 and 7 showed a decrease in LCMV titers, it is likely that an increase in the number of treatments after days 6 and 7 will provide a further decrease in viral load. Alternatively, or in addition, the amount of PapMV VLPs administered could be increased, for example, to 200 μg per dose.

[0199] Viral titers found in various organs of the animals are shown in FIG. **19**. While the viral load in the brain of animals treated at days 6 and 7 (Group B) showed a significant reduction, viral loads in other organs of the treated mice did not show a significant reduction. This is most likely because the titers were measured at day 15, which allowed the infection sufficient time to 'kick back' after treatment. Subsequent treatments to days 6 and 7 would be anticipated to lead to a more significant decrease in viral loads.

Example 14

Multiple Treatments with PapMV VLPs Prolong the Protection Period

[0200] Example 2 demonstrates that the protection induced by the treatment with PapMV VLPs appears to persist for a period of about 5 days. To investigate if treatment with multiple doses of PapMV VLPs could provide a longer period of protection, mice were treated following the general regimen described in Example 1 with PapMV VLPs containing ssRNA once (1×), twice (2×), 5 times (5×) or 10 times (10×) at 1-week intervals. Three days after the final treatment, the mice were challenged with influenza WSN/33 virus as described in Example 2.

[0201] The weight loss of the mice is shown in FIG. **20**. It was also noted that the animals were not affected by the multiple treatments and gained weight normally during the treatment period in line with the control animals.

[0202] These results show that multiple treatments can extend the period of protection induced by the PapMV VLP nanoparticles to more than 10 weeks. The results also demonstrate that multiple treatments with PapMV VLPs do not exhaust the innate immunity of the animal. Finally, as it is known that antibodies to the PapMV VLPs appear 7 days after the first treatment and increase with the booster treatments, these results demonstrate that the ability of the PapMV VLPs to trigger the innate immune response is not impacted by the production of antibodies.

Example 15

Induction of Neutrophil Recruitment by PapMV VLPs

[0203] Mice were submitted to 2 instillations of PapMV VLPs containing ssRNA according to the protocol of Example 1 and broncho-alveolar lavage (BAL) was performed 6 hours after the second treatment. The results are shown in FIG. **21**. Neutrophils found into the BAL of mice treated with PapMV VLPs are circled in FIG. **21**B. Three times more neutrophils were observed in the treated mice compared to the control group.

[0204] Neutrophils represent the first line of defense. This Example demonstrates that neutrophils are recruited rapidly in mice treated with PapMV VLPs; just 6 hours after treatment. Neutrophils are known to play a key role in the control of bacterial and viral infection in the lungs and thus likely play a role in the protection observed in PapMV treated mice.

Example 16

Induction of a Mucosal Immune Response by PapMV VLPs

[0205] Balb/C mice (10 per group) were treated with two instillations of 20 μg PapMV VLP ssRNA combined with 2 μg of the trivalent inactivated flu vaccine (TIV) at 14 day intervals. Bleedings were performed at day 0, 14 and 28. Following the same protocol, another group of mice were immunized animals by the s.c. route for comparison. Mice were challenged at day 15 with $1LD_{50}$ of the influenza WSN/33 virus and weight loss was followed over a 14 day period.

[0206] IgG titers were measured in the blood of the immunized animals by ELISA using antibodies to the TIV and the results are shown in FIG. **22**. The addition of PapMV VLPs to the TIV increased significantly the total IgG and the IgG2a response as compared with the group immunized with TIV alone when the same route of immunization was used. Interestingly, the s.c. route was more efficient than the i.n. route for production of total IgG and IgG2a in the blood of the animal.

[0207] Antibody titers were measured in the broncho-alveolar lavage (BAL) and in the faeces of the immunized animals by ELISA using antibodies to the TIV and the results are shown in FIG. **23**. From these results, it is clear that only i.n. treatment triggers production of IgA in the BAL. The addition of PapMV VLPs to the TIV increased significantly the amount of IgA in the lungs as compared to instillation with TIV alone. Significantly higher total IgG in the BAL was also observed in the animals treated with PapMV VLPs in combination with the TIV, as compared to the TIV alone group. The amount of total IgG in the BAL obtained from mice treated intranasally with PapMV VLPs in combination with the TIV administered by i.n. was not significantly different from that in mice treated subcutaneously with the combination. Finally, it was interesting to note that a mucosal immune response was also observed in the intestines of the mice treated intranasally with the combination as shown by the presence of IgA directed to TIV in this organ (FIG. **23**C).

[0208] Weight loss in the mice after challenge with the influenza virus is shown in FIG. **24**. The challenge revealed that immunization by the intranasal route is more robust and efficient in protecting mice to a heterosubtypic strain than immunization by the s.c. route. In the group immunized by the i.n. route with the combination of PapMV VLPs and TIV,

the mice gained weight and did not show any symptoms. The combination administered s.c. provided only a partial protection. Complete protection can, however, be achieved using s.c. administration of 3 μg of TIV with 30 μg of PapMV VLPs. All the other groups that were immunized with TIV alone (by either route), PapMV VLPs alone or the control buffer were not protected, showed symptoms of disease and lost significant amounts of weight.

[0209] The results from this experiment demonstrate that PapMV VLPs can act as a mucosal adjuvant. The ability of an adjuvant to trigger a mucosal immune response is important for effective prevention or treatment of infections and diseases caused by micro-organisms that gain access to the body via mucosal membranes, including influenza, tuberculosis, and *H. pylori* infections. The presence of IgG in the faeces of the immunized animals suggests that i.n. vaccinations using PapMV VLPs as adjuvant could be used to protect against bacterial or viral infection in the intestine. In addition, since the mucosal immune response triggered by the PapMV VLPs is general, i.n. vaccinations using PapMV VLPs as adjuvant could potentially also be used to protect against bacterial or viral infection (such as HIV-1) in the vaginal mucosa.

[0210] Although in this experiment no protection was seen in mice treated i.n. with PapMV VLPs alone, this is consistent with the results in the previous examples which indicate that the non-specific protection induced by PapMV VLPs lasts only for a period of about 5 days. In this experiment, the challenge was performed 14 days after the second instillation of VLPs.

Example 17

Activation of TLR-2 and CD14 by PapMV VLPs

[0211] As demonstrated in the preceding Examples, PapMV VLPs prepared in bacterial host cells and PapMV VLPs prepared by in vitro self-assembly with ssRNA are both able to stimulate the innate immune response. However, VLPs prepared by the two different methods, activate different TLRs. As shown above, PapMV VLPs prepared by in vitro self-assembly with ssRNA activate TLR-7. In contrast, PapMV VLPs prepared by expression of the PapMV coat protein and self-assembly in *E. coli* cells as previously described (Tremblay et al., 2006, ibid.), activate TLR-2 and CD14.

[0212] In brief, THP1-XBlue™-CD14 cells (InvivoGen, San Diego, Calif.) were treated with 100 μg PapMV VLPs (prepared according to Tremblay et al.) or a known TLR ligand (100 μg lipotcichoic acid from *S. aureus* (LTA): TLR2 and CD14 ligand; 1 μg Pam3SCK4: TLR2 ligand; or 10 μg flagellin: TLR5 ligand) and either an anti-CD14, anti-TLR2 or anti-TLR5 antibody. THP1-XBlue™-CD14 cells harbour several TLRs (including TLR2, 4, 5) and have been modified to produce a blue colour when a TLR is engaged with a ligand. Upon engagement, the cells become blue and the strength of the engagement can be readily evaluated using a spectrophotometer. Measurements were made after a 24 hour incubation of the cells at 37° C.

[0213] The results are shown in FIG. **26**. Antibodies (Ac) directed to CD14, TLR2 or TLR5 blocked engagement of the respective TLR or CD14 and revealed what interactions were being made by each test molecule. A significant decrease in optical density was observed when the anti-TLR2 antibody was used with the PapMV VLPs, and a strong decrease observed when the anti-CD 14 antibody was used. In this experiment, the antibody to TLR2 did not work as well as expected as a higher decrease should have been observed when Pam3CSK4 (a known TLR2 ligand) was used. It is likely the amount of Pam3CSK4 used in the experiment was too high.

[0214] The difference in TLR activation seen with the PapMV VLPs assembled in bacteria may be due to the detergent treatment that the VLPs undergo after isolation from the bacterial cells. This treatment may affect the surface of the PapMV VLPs, for example to expose hydrophobic residues, and result in the VLPs becoming a ligand of TLR2. In contrast to TLR7, which is present in the endosome, both TLR2 or CD14 are surface exposed on immune cells.

Example 18

Method for Preparing PapMV VLPs Comprising ssRNA

[0215] This example describes a process for preparing PapMV VLPs in vitro by assembling recombinant PapMV Coat Protein (rCP) onto synthetic RNA templates (SRT) to produce recombinant VLPs (rVLPs). The rVLPs are rod shaped nanoparticles 15 nm wide, and 50 to thousands nm in length. The process is summarized in the flow chart presented in FIG. **27**.

Production of rCP

[0216] rCP harbouring a 6×His-tag was produced in *E. coli* transformed with plasmid DNA containing the rCP gene under the control of an inducible promoter. In brief, the PapMV CP was cloned into the pQE80 vector (QIAGEN) flanked by the restriction enzyme NcoI and BamHI and the protein was expressed under the control of the T5 promoter. *E. coli* BD-792 was used for expression. Transformed bacteria were grown in standard culture medium. Protein expression was triggered by addition of a biochemical inducer to the culture medium (0.7-1 mM IPTG for 6-9 h at 22-25° C.). At the end of the induction period, cells were harvested, suspended in lysis buffer (10 mM Tris pH 8.0, 500 mM NaCl) and ruptured mechanically using a French press, homogenizer or sonicator. Cell lysate was clarified by removal of genomic DNA by standard DNase treatment and removal of large cell fragments and membranes by centrifugation or tangential flow filtration (300 kDa to 0.45 μm MWCO membranes). rCP was captured on an ion-matrix affinity resin and eluted with a pH gradient or imidazole using standard procedures. The rCP was subsequently purified from endotoxins by anion exchange chromatography/filtration and from small low MW molecules by tangential flow filtration (0 to 30 kDa MWCO membranes). Any contaminating imidazole present in the rCP solution was removed by dialysis or tangential flow filtration (5 to 30 kDa MWCO membranes). The final rCP protein solution was sterilized by filtration. The sterile product can be stored at 2-8° C. and is stable for several years.

Production of SRT

[0217] The sequence of the SRT is provided in FIG. **28** [SEQ ID NO:5]. The SRT is based on the genome of PapMV and harbours the PapMV coat protein nucleation signal at the 5'-end (boxed in FIG. **28**). The remaining nucleotide sequence is poly-mutated in that all ATG codons have been mutated for TAA stop codons. The first 16 nucleotides of the sequence (underlined in FIG. **28**) comprise the T7 transcrip-

tion start site located within the pBluescript expression vector and are present within the RNA transcript. Pentameric repeats are underlined in FIG. **28**. The entire transcript is 1522 nucleotides in length.

[0218] DNA corresponding to the SRT was inserted into a DNA plasmid including a prokaryotic RNA polymerase promoter using standard procedures. The recombinant plasmid was used to transform *E. coli* cells and the transformed bacteria were subsequently grown in standard culture medium. The plasmid DNA was recovered and purified from the cell culture by standard techniques, then linearized by cleavage with a restriction enzyme (MluI) at the point in the DNA sequence immediately after the last nucleotide that was to be present in SRT.

[0219] Transcription of SRT was conducted with T7 RNA polymerase using the RiboMAX™ kit (Promega, USA) following the manufacturer's recommended protocol. The expression vector was designed such that transcripts originating from the RNA polymerase promoter were released from the DNA template at the DNA point of cleavage. The SRT was purified to remove DNA, protein and free nucleotides by tangential flow filtration using a 100 kDa MWCO membrane. The final RNA solution was sterilized by filtration. The sterile product can be stored below −60° C. and is stable for several years.

Production of rVLPs

[0220] rVLPs were assembled in vitro by combining the rCP and SRT. The assembly reaction was conducted in a neutral buffered solution (10 mM Tris-HCl pH 8). The assembly reaction was conducted using a protein:RNA ratio between 15-30 mg of protein for 1 mg RNA. The newly assembled rVLPs were incubated with a low amount of RNase (0.0001 μg RNAse per μg RNA) to remove any RNA protruding from the rVLPs. This step improves the solubility of the rVLPs. The blunted-rVLPs were then purified from contaminants and free rCP (unassembled monomeric rCP) by diafiltration using 10-100 kDa MWCO membranes. The final rVLP liquid suspension was sterilized by filtration. The sterile product can be stored at 2-8° C. and is stable for several years.

[0221] The disclosure of all patents, publications, including published patent applications, and database entries referenced in this specification are expressly incorporated by reference in their entirety to the same extent as if each such individual patent, publication, and database entry were expressly and individually indicated to be incorporated by reference.

[0222] Although the invention has been described with reference to certain specific embodiments, various modifications thereof will be apparent to those skilled in the art without departing from the spirit and scope of the invention. All such modifications as would be apparent to one skilled in the art are intended to be included within the scope of the following claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 6

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<211> LENGTH: 215
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<213> ORGANISM: Papaya mosaic Virus (PapMV)

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            20                  25                  30

Leu Pro Ser Gln Glu Gln Leu Lys Ser Val Ser Thr Leu Met Val Ala
        35                  40                  45

Ala Lys Val Pro Ala Ala Ser Val Thr Thr Val Ala Leu Glu Leu Val
    50                  55                  60

Asn Phe Cys Tyr Asp Asn Gly Ser Ser Ala Tyr Thr Thr Val Thr Gly
65                  70                  75                  80

Pro Ser Ser Ile Pro Glu Ile Ser Leu Ala Gln Leu Ala Ser Ile Val
                85                  90                  95

Lys Ala Ser Gly Thr Ser Leu Arg Lys Phe Cys Arg Tyr Phe Ala Pro
            100                 105                 110

Ile Ile Trp Asn Leu Arg Thr Asp Lys Met Ala Pro Ala Asn Trp Glu
        115                 120                 125

Ala Ser Gly Tyr Lys Pro Ser Ala Lys Phe Ala Ala Phe Asp Phe Phe
    130                 135                 140

Asp Gly Val Glu Asn Pro Ala Ala Met Gln Pro Pro Ser Gly Leu Ile
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```

-continued

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Arg Ser Pro Thr Gln Glu Glu Arg Ile Ala Asn Ala Thr Asn Lys Gln
                165                 170                 175

Val His Leu Phe Gln Ala Ala Ala Gln Asp Asn Asn Phe Thr Ser Asn
            180                 185                 190

Ser Ala Phe Ile Thr Lys Gly Gln Ile Ser Gly Ser Thr Pro Thr Ile
        195                 200                 205

Gln Phe Leu Pro Pro Pro Glu
    210                 215


<210> SEQ ID NO 2
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
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tcagtgtcca ccctcatggt agctgctaag gttccagcag ccagtgttac aactgtggca    180

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acttccctta gaaaattctg ccggtacttc gcgccaataa tctggaatct gaggacggac    360

aaaatggctc ctgccaattg ggaggcttca ggatacaagc caagcgccaa atttgccgcg    420

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aggtcgccga cccaggaaga gcggattgcc aatgctacca acaaacaggt gcatctcttc    540

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<213> ORGANISM: Artificial sequence
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Met Ser Ser Ile Lys Val Asp Pro Thr Ser Asn Leu Leu Pro Ser Gln
            20                  25                  30

Glu Gln Leu Lys Ser Val Ser Thr Leu Met Val Ala Ala Lys Val Pro
        35                  40                  45

Ala Ala Ser Val Thr Thr Val Ala Leu Glu Leu Val Asn Phe Cys Tyr
    50                  55                  60

Asp Asn Gly Ser Ser Ala Tyr Thr Thr Val Thr Gly Pro Ser Ser Ile
65                  70                  75                  80

Pro Glu Ile Ser Leu Ala Gln Leu Ala Ser Ile Val Lys Ala Ser Gly
                85                  90                  95

Thr Ser Leu Arg Lys Phe Cys Arg Tyr Phe Ala Pro Ile Ile Trp Asn
            100                 105                 110

Leu Arg Thr Asp Lys Met Ala Pro Ala Asn Trp Glu Ala Ser Gly Tyr
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Lys Pro Ser Ala Lys Phe Ala Ala Phe Asp Phe Phe Asp Gly Val Glu
    130                 135                 140

Asn Pro Ala Ala Met Gln Pro Pro Ser Gly Leu Thr Arg Ser Pro Thr
145                 150                 155                 160

Gln Glu Glu Arg Ile Ala Asn Ala Thr Asn Lys Gln Val His Leu Phe
                165                 170                 175

Gln Ala Ala Ala Gln Asp Asn Asn Phe Ala Ser Asn Ser Ala Phe Ile
            180                 185                 190

Thr Lys Gly Gln Ile Ser Gly Ser Thr Pro Thr Ile Gln Phe Leu Pro
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Pro Pro Glu
    210


<210> SEQ ID NO 4
<211> LENGTH: 222
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
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            20                  25                  30

Glu Gln Leu Lys Ser Val Ser Thr Leu Met Val Ala Ala Lys Val Pro
        35                  40                  45

Ala Ala Ser Val Thr Thr Val Ala Leu Glu Leu Val Asn Phe Cys Tyr
    50                  55                  60

Asp Asn Gly Ser Ser Ala Tyr Thr Thr Val Thr Gly Pro Ser Ser Ile
65                  70                  75                  80

Pro Glu Ile Ser Leu Ala Gln Leu Ala Ser Ile Val Lys Ala Ser Gly
                85                  90                  95

Thr Ser Leu Arg Lys Phe Cys Arg Tyr Phe Ala Pro Ile Ile Trp Asn
            100                 105                 110

Leu Arg Thr Asp Lys Met Ala Pro Ala Asn Trp Glu Ala Ser Gly Tyr
        115                 120                 125

Lys Pro Ser Ala Lys Phe Ala Ala Phe Asp Phe Phe Asp Gly Val Glu
    130                 135                 140

Asn Pro Ala Ala Met Gln Pro Pro Ser Gly Leu Thr Arg Ser Pro Thr
145                 150                 155                 160

Gln Glu Glu Arg Ile Ala Asn Ala Thr Asn Lys Gln Val His Leu Phe
                165                 170                 175

Gln Ala Ala Ala Gln Asp Asn Asn Phe Ala Ser Asn Ser Ala Phe Ile
            180                 185                 190

Thr Lys Gly Gln Ile Ser Gly Ser Thr Pro Thr Ile Gln Phe Leu Pro
        195                 200                 205

Pro Pro Glu Thr Ser Thr Thr Arg His His His His His His
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<213> ORGANISM: Artificial sequence
<220> FEATURE:
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<223> OTHER INFORMATION: ssRNA template

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gataaggtgg taataacact gacctaccca acaaaaactt ttgacaatcg gggttgggtg 1380

gttaagctgg aagcgaggag gggttacgag tggttcgctg taaagtaaca caagccgaag 1440

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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
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28. (canceled)
29. (canceled)
30. (canceled)
31. (canceled)

32. A method of stimulating an innate immune response in a subject comprising administering to the subject an effective amount of a composition comprising papaya mosaic virus (PapMV) or PapMV virus-like particles (VLPs), thereby preventing, or decreasing the severity of, a microbial infection in the subject.

33. The method according to claim **32**, wherein the microbial infection is an infection with a microorganism that gains access to the subject's body via mucosal membranes.

34. The method according to claim **32**, wherein the microbial infection is a viral infection.

35. The method according to claim **32**, wherein the microbial infection is a bacterial infection.

36. The method according to claim **32**, for preventing a microbial infection in the subject.

37. The method according to claim **32**, for decreasing the severity of a microbial infection in the subject.

38. The method according to claim **32**, wherein the immune response is stimulated at a mucosal surface.

39. The method according to claim **38**, wherein the composition is administered in combination with one or more antigens.

40. The method according to claim **32**, wherein the composition is administered via an intranasal route.

41. The method according to claim **32**, wherein the composition comprises PapMV.

42. The method according to claim **32**, wherein the composition comprises PapMV VLPs.

43. The method according to claim **42**, wherein the PapMV VLPs comprise ssRNA.

44. A method of protecting a subject against infection with a pathogen comprising administering to the subject an effective amount of a composition comprising papaya mosaic virus (PapMV) or PapMV virus-like particles (VLPs), wherein the composition stimulates the innate immune response in the subject.

45. The method according to claim **44**, wherein the pathogen is a microorganism that gains access to the subject's body via mucosal membranes.

46. The method according to claim **44**, wherein the pathogen is a virus.

47. The method according to claim **46**, wherein the virus is an influenza virus, flavivirus, parainfluenza virus, respiratory syncytial virus, coronavirus, adenovirus or rhinovirus.

48. The method according to claim **44**, wherein the pathogen is a bacterium.

49. The method according to claim **48**, wherein the bacterium is *Bacillus anthracis, Yersinia pestis, Francisella tularensis, Streptococcus pnemoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Burkholderia cepacia, Corynebacterium diphtheriae, Legionella pneumophila, Mycoplasma pneumoniae, Chlamydophila pneumoniae, Mycobacterium tuberculosis, Moraxella catarrhalis, Haemophilus influenzae, Klebsiella pneumoniae, Escherichia coli* or *Coxiella burnetii*.

50. The method according to claim **44**, wherein the composition is administered to the subject between 4 hours and 6 days prior to expected exposure of the subject to the pathogen.

51. The method according to claim **44**, wherein the composition is administered to the subject in multiple doses.

52. The method according to claim **44**, wherein the subject is immunocompromised, undergoing chemotherapy or taking immunosuppressive drugs.

53. The method according to claim **44**, wherein the composition prevents immune exhaustion in the subject.

54. The method according to claim **44**, wherein the composition is administered via an intranasal route.

55. The method according to claim **44**, wherein the composition comprises PapMV.

56. The method according to claim **44**, wherein the composition comprises PapMV VLPs.

57. The method according to claim **56**, wherein the PapMV VLPs comprise ssRNA.

58. A method of treating a chronic or recurrent microbial infection comprising administering to a subject having a chronic or recurrent microbial infection a composition comprising Papaya Mosaic Virus (PapMV) or PapMV virus-like particles (VLPs).

59. The method according to claim **58**, wherein the composition is administered in combination with one or more conventional therapies.

60. The method according to claim **58**, wherein the microbial infection is a bacterial infection.

61. The method according to claim **58**, wherein the microbial infection is a viral infection.

62. The method according to claim **61**, wherein the viral infection is a hepatitis C virus infection or human immunodeficiency virus infection.

63. The method according to claim **61**, wherein the composition decreases viral load in the subject.

64. The method according to claim **58**, wherein the composition is administered via an intranasal route.

65. The method according to claim **58**, wherein the composition comprises PapMV.

66. The method according to claim **58**, wherein the composition comprises PapMV VLPs.

67. The method according to claim **66**, wherein the PapMV VLPs comprise ssRNA.

68. A kit comprising a container having contained therein a pharmaceutical composition comprising papaya mosaic virus (PapMV) or PapMV virus-like particles (VLPs), the container adapted to deliver the pharmaceutical composition by an intranasal, pulmonary or vaginal route.

69. The kit according to claim **68**, wherein the container is an inhaler, nebulizer or nasal spray device.

* * * * *