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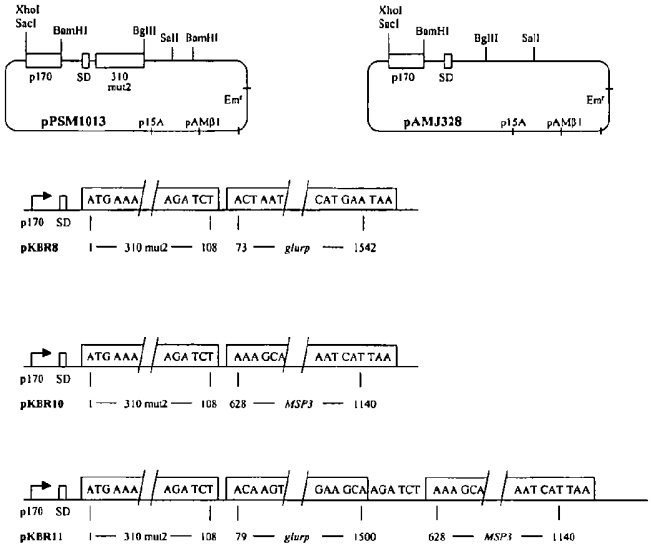
(54) Benævnelse: Vaccine comprising chimeric malaria proteins derived from Plasmodium falciparum

(57) Sammendrag:

A fusion protein, derived from *P. falciparum* Glutamate-rich protein (GLURP) genetically coupled to *P. falciparum* Merozoite surface protein 3 (MSP3) was produced in *Lactococcus lactis* as a secreted recombinant GLURP-MSP3 hybrid protein and experiments showed that the GLURP-part of the hybrid increased the overall antibody response. Immunizations with the hybrid protein consistently generated a stronger antibody response against the individual GLURP and MSP3 domains than a mixture of the two recombinant molecules injected at one site or the individual recombinant molecules injected simultaneously at two different sites. The difference was most pronounced for the MSP3-specific antibody response suggesting that T cell epitopes located in the GLURP R0-region provide help for B-cell epitopes in the MSP3 region. Moreover, when the animals were injected with a mixture of GLURP and MSP3, individual mice tended to mount a predominant antibody response against either molecule: in some animals GLURP was immuno-dominant whereas in other animals MSP3 was the dominant immunogen. Additionally, the hybrid was also more antigenic than the individual recombinant proteins since the ELISA-titer of naturally occurring IgG antibodies, in clinically immune African adults, against the hybrid protein was higher than the titers against the individual recombinant proteins. The hybrid protein was also demonstrated to be a potential protective antigen as mouse anti-GLURP-MSP3 IgG antibodies were able to inhibit parasite-growth *in vitro* in a monocyte-dependent manner.

fortsættes


XhoI
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SalI
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SD
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mut2
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XhoI
SacI
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BglII
SalI
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Em^r
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pAMβ1
ATG AAA
AGA TCT
ACT AAT
CAT GAA TAA
p170 SD
pKBR8 1 — 310 mut2 — 108 73 — glurp — 1542
ATG AAA
AGA TCT
AAA GCA
AAT CAT TAA
p170 SD
pKBR10 1 — 310 mut2 — 108 628 — MSP3 — 1140
ATG AAA
AGA TCT
ACA AGT
GAA GCA
AGA TCT
AAA GCA
AAT CAT TAA
p170 SD
pKBR11 1 — 310 mut2 — 108 79 — glurp — 1500 628 — MSP3 — 1140

Claims

1. An antigen based vaccine against malaria comprising a fusion protein derived from *Plasmodium falciparum* Glutamate-rich protein (GLURP) genetically coupled to at least one other *Plasmodium falciparum* derived protein or a homologue of said fusion protein.

2. An antigen based vaccine against malaria according to claim 1 where the protein genetically coupled to GLURP is derived from the Merozoite surface protein 3 (MSP3) from *Plasmodium falciparum*.

3. A vaccine according to claim 2 comprising SEQ ID NO 1

4. A vaccine according to claim 2 or 3 further comprising an immunogenic fragment of a protein derived from *Plasmodium falciparum*

5. A fusion protein comprising SEQ ID NO. 1 or a homologue hereof.

6. A fusion protein according to claim 5 further comprising one or more immunogenic fragments of one or more proteins derived from *Plasmodium falciparum.*

7. A fusion protein according to claim 6 where the immunogenic fragment is chosen from CS, MSP1, MSP2, MSP4, MAP5, MSP6, AMA1, Pf155/RESA, RAP1, EBA-175, EMP1, EXP1, LSA1, LSA3, Pf25, Pf45/48, Pf230, Pf27, Pf16, or Pf28.

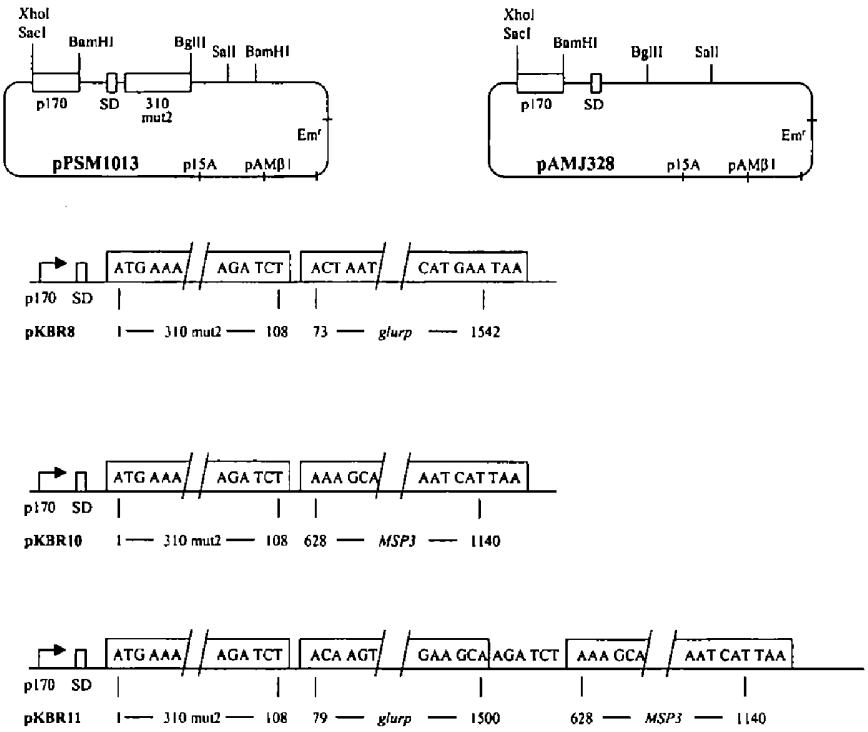
8. Preparation of a fusion protein according to claim 5-7 from a recombinant *Lactococcus sp.*

9. A nucleic acid comprising SEQ ID NO. 2 or a homologue hereof.

10. A nucleic acid coding for a fusion protein according to claim 6 or 7

11. Use of a nucleic acid according to claim 9 or 10 for the preparation of a vaccine.

12. A vaccine comprising a recombinant BCG expressing the nucleic acid sequence according to claim 9 or 10.


XhoI
SacI
BamHI
BglII
SalI
BamHI
p170
SD
310
mut2
Em^r
pPSM1013
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pAMβ1
XhoI
SacI
BamHI
BglII
SalI
p170
SD
Em^r
pAMJ328
p15A
pAMβ1
ATG AAA
AGA TCT
ACT AAT
CAT GAA TAA
p170 SD
pKBR8 1 — 310 mut2 — 108 73 — glurp — 1542
ATG AAA
AGA TCT
AAA GCA
AAT CAT TAA
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AGA TCT
ACA AGT
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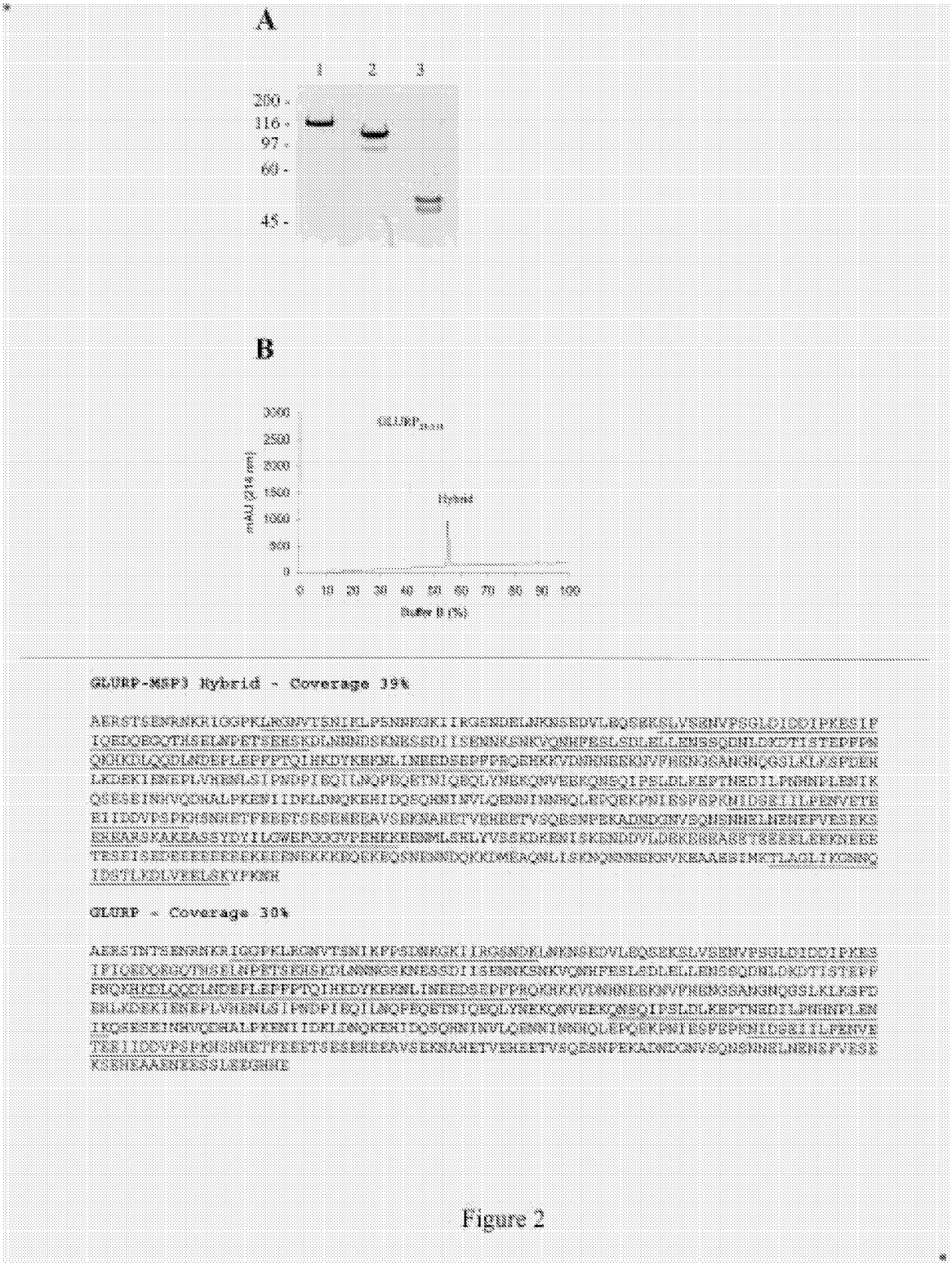
p170 SD
pKBR11 1 — 310 mut2 — 108 79 — glurp — 1500 628 — MSP3 — 1140

Figure 1



GLURP-MSP3 Hybrid - Coverage 39%

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IQEDQDQQTNSELNPETSEESKDLNNNDSKNESSDIISENNKSNKVQNNPESLSDLELLENSSQDNLDKDTISTEPFPN
QPNKDLQQDLNDEPLEPFPTQIHKDYKEKNLINEEDSEPFPRQKHKKVTNNNEEKNVFNENGSANGNQGSLKLKSPD
LKDEKINEPLVNENLSIPNDPIEQILNQPEQETNIQEQLYNEKQNVEEEQNSQIPSLDLEEPTNEDILPNNNPLENIK
QSESEINNVQDNALPKENIIDKLDNQKENIDQSQNNINVLQENNINNNQLEPQEKPNIESVPEPKNIDSEIILPENVETE
EIIIDDVPSPKNSNNETFEEETSESENEEAVSENNANETVEHEETVSQESNPEKADNDGNVSQNSNNELNENEPVESEKS
ENEARSKAKEASSYDYILGWEPQRDVPENKEENNMLSHLYVSSKDKENISKENDDVLDRKERRANSTSSESLEEDNEEE
TESEISEDEEEEEEEEEKEEENEKKEDQEKEQSNEGNDQKKIMEAQNLISKNQNNNEDNVKEAAEESIMSTLAGLIKGNSQ
IDSTLEDLVFELSKYFKNN

GLURP - Coverage 30%

AERSTNTSENRNKRIGGPKLRGNVTSNIKPPSDNRGKIIRGSNDELNKNSEDVLEQSEKSLVSENVPSGLDIDDIPKES
IFIQEDQDQQTNSELNPETSNNSFDLNNNGSKNESSDIISENNKSNKVQNNPESLSDLELLENSSQDNLDKDTISTEPF
FNQPNKELQQDLNDEPLEPFPTQIHKDYKEKNLINEEDSEPFPRQKHKKVTNNNEEKNVFNENGSANGNQGSLKLKSPD
ELLKDEKINEPLVNENLSIPNDPIEQILNQPEQETNIQEQLYNEKQNVEEKQNSQIPSLDLEEPTNEDILPNNNPLEN
IKQSEESEINNVQDNALPKENIIDKLDNQKENIDQSQNNINVLQENNINNNQLEPQEKPNIESVPEPKNIDSEIILPENVE
TEEIIDDVPSPKNSNNETFEEETSESENEEAVSENNANETVEHEETVSQESNPEKADNDGNVSQNSNNELNENSPVESS
KGENEAADNEESSLEEDNNE

Figure 2

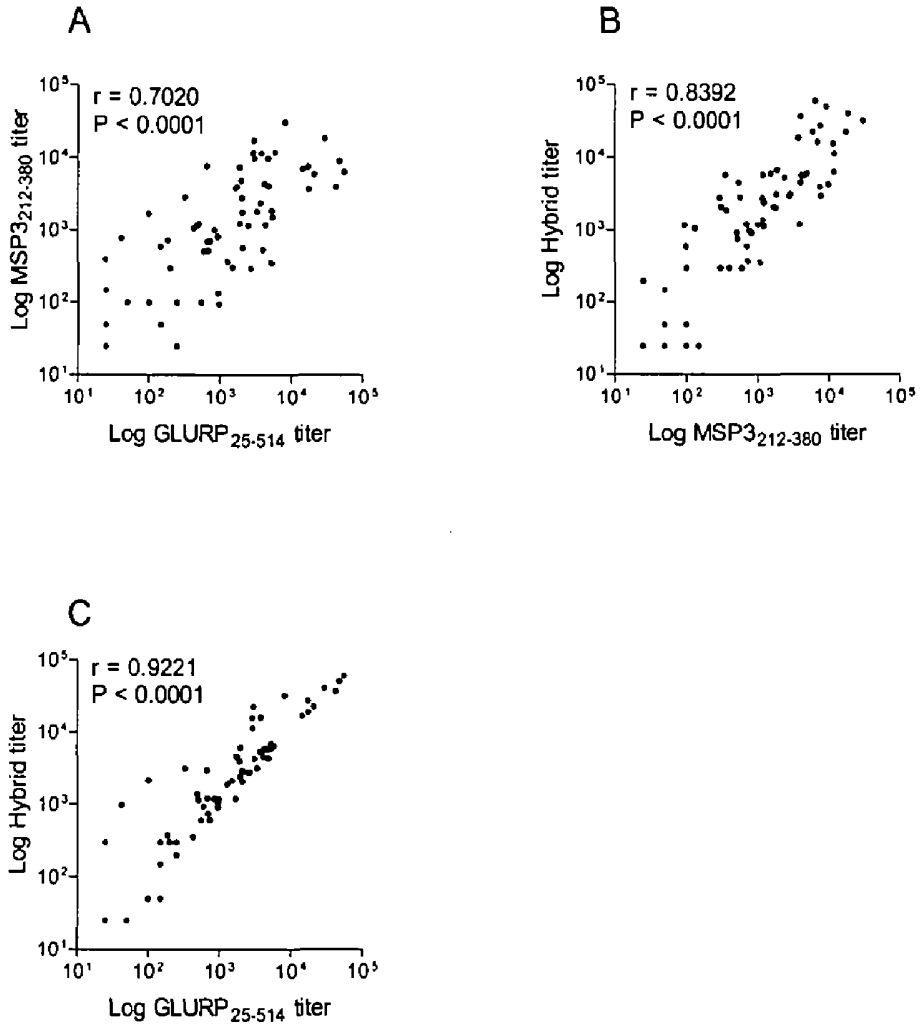
A
r = 0.7020
P < 0.0001
Log MSP3212-380 titer
Log GLURP25-514 titer
B
r = 0.8392
P < 0.0001
Log Hybrid titer
Log MSP3212-380 titer
C
r = 0.9221
P < 0.0001
Log Hybrid titer
Log GLURP25-514 titer

Figure 3

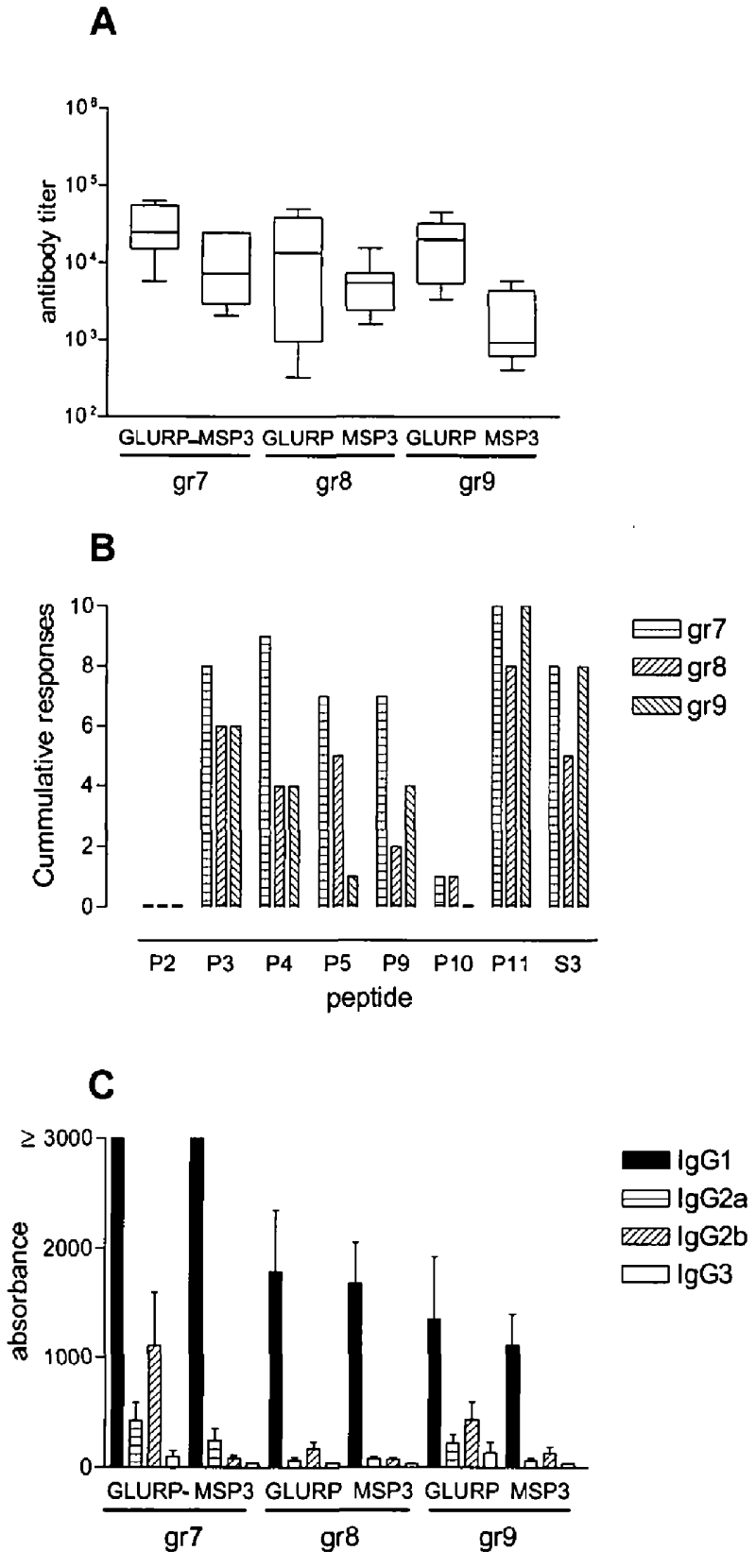
A
antibody titer
GLURP-MSP3 GLURP MSP3 GLURP MSP3
gr7 gr8 gr9
B
Cummulative responses
gr7
gr8
gr9
P2 P3 P4 P5 P9 P10 P11 S3
peptide
C
absorbance
IgG1
IgG2a
IgG2b
IgG3
GLURP-MSP3 GLURP MSP3 GLURP MSP3
gr7 gr8 gr9

Figure 4

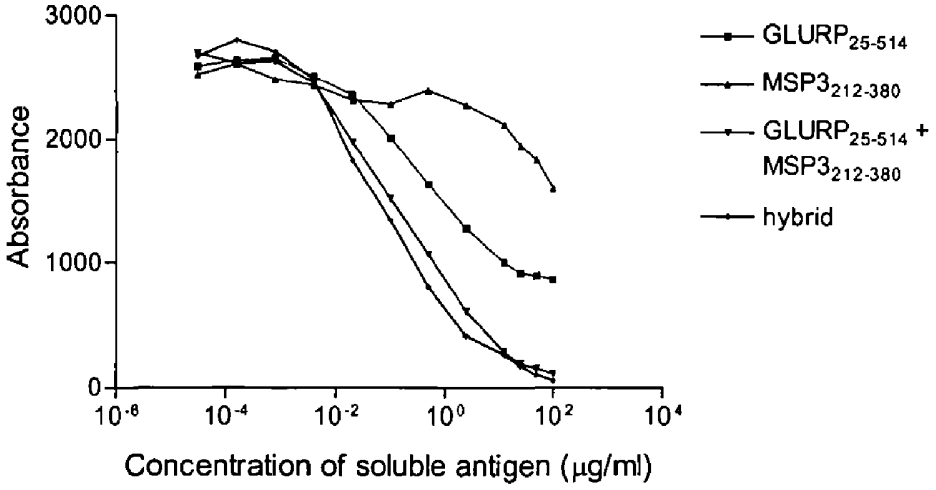
3000
2000
1000
0
Absorbance
10^{-6}
10^{-4}
10^{-2}
10^{0}
10^{2}
10^{4}
Concentration of soluble antigen (µg/ml)
GLURP25-514
MSP3212-380
GLURP25-514 + MSP3212-380
hybrid

Figure 5

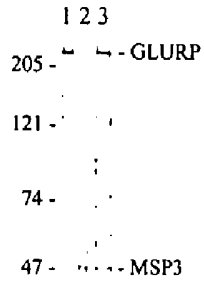

Figure 6