Hepatitis B core as a VLP carrier

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Bethesda, September 22, 2009
VLPs = icosahedrons (viral cores)
Icosahedron (viral core) with outer envelope (surface)
A typical example of an enveloped virus with icosahedral core: Hepatitis B virus
Hepatitis B surface: RTS,S malaria vaccine

Evaluation of RTS,S/AS02A and RTS,S/AS01B in Adults in a High Malaria Transmission Area

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Expert Review of Vaccines
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(doi:10.1586/14760584.8.3.265)

Key Paper Evaluation
Malaria vaccine: the latest news from RTS,S/AS01E vaccine
Gloria Gonzalez-Aseguinolaza


The development of the RTS,S malaria vaccine candidate: challenges and lessons.
Ballou WR.
Infectious Diseases Development, Global Health Division, Bill & Melinda Gates Foundation, PO Box 23350, Seattle, WA, USA. rip.ballou@gatesfoundation.org


A vaccine against malaria: a substantial step forward.
Moorthy V, Smith PG, Kieny MP.
Initiative for Vaccine Research, Department of Immunization, Vaccines and Biologicals, WHO, 1211 Geneva 27, Switzerland. vasee.moorthy@gmail.com
Hepatitis B core VLPs: Packaging

Oligonucleotides, DNA, RNA
Proteins
Peptides
Drugs

Vaccines
Drug delivery
Gene therapy
Chemotherapy
VLPs: Combination of Exposure and Packaging

Oligonucleotides, DNA, RNA
Proteins
Peptides
Drugs

Vaccines
Drug delivery
Gene therapy
Chemotherapy
VLPs: Nanotechnological Applications

Nanomagnetic particles

Physics
Electronics
Types of VLPs

- **non-infectious empty**
- **infectious replication-competent**
- **infectious replication-noncompetent**
3D structure of the HBc platform
Human Hepatitis B Viral Capsid (Hbcag)

PDB_ID: 1QGT


Molecular Cell 3 pp. 771 (1999)
HBc: Electron microscopy – 34 nm (T=4) and 30 nm (T=3)
HBc on the size scale of the most promising VLP candidates
Hepatitis B core: T=4 and T=3

Vaccines:
- HBV
- HCV
- Malaria
- Influenza

http://viperdb.scripps.edu/
Hepatitis B core as a platform
Structural and immunological features of the HBc protein

monomer  tetramer - asymmetric unit

B epitopes  CTL epitopes
HBc tetramer: putative insertion sites

MIR: positions 79-81

C-terminal: positions 144, 161, 167, etc

Wynne et al., 1999
HBc tetramer (surface): putative insertion sites

C-terminal: positions 144, 161, 167, etc

MIR: positions 79-81

Wynne et al., 1999
HBc dimer (chains A and B - surface): putative insertion sites

MIR: positions 78-81

C-terminal: positions 142-143
HBc dimer (chains A and B): putative insertion sites

MIR: positions 78-81

C-terminal: positions 142-143
Chimeric HBc dimer (chains A and B): “successful” insertion at the MIR

modelling by 3D-JIGSAW

http://bmm.cancerresearchuk.org/~3djigsaw/
Chimeric HBc dimer (A and B - surface): “successful” insertion at the MIR

MIR: insertion at the position 78

modelling by 3D-JIGSAW

http://bmm.cancerresearchuk.org/~3djigsaw/
Protein engineering on the HBc
HBc VLPs: Expression

Promoter

HBc gene

Virus-like particles (VLPs)

- *Escherichia coli*
- *Pichia pastoris*
- *Hansenula polymorpha*
Construction of chimeric HBc VLPs: direct gene fusion
Construction of chimeric HBc VLPs: direct gene fusion
Construction of Chimeric HBc VLPs: Chemical Coupling

http://www.cytos.com

Martin F. Bachmann
Zuerich
HBc: design

DNA/RNA packaging:
- gene therapy

Surface elements:
- vaccines
- diagnostics
- gene therapy
HBc VLPs: Types of chimeric particles

- Uniform
- Mosaic
- Mosaic multi-epitope
HBc: what and where to insert?
Promoter

Surface elements

Surface elements

Hidden elements

Up to 250 foreign aa

Up to 50 foreign aa

Up to 800 foreign aa

HBc: capacity
HBc vectors
Main problems of the HBc: capacity and exposition

VECTOR CLASSES: MIR

full-length

C-terminally truncated

exposed: YES  capacity: ?  rate of success: ?

major immunodominant region (MIR) aa 79-81
Main problems of the HBc: capacity and exposition

VECTOR CLASSES: N-terminus

exposed: ?
capacity: ?
rate of success: ?
Main problems of the HBc: capacity and exposition

VECTOR CLASSES: C-terminus

full-length

C-terminally truncated

exposed: NO  capacity: HIGH  rate of success: HIGH
Main problems of the HBc: capacity and exposition

VECTOR CLASSES: C-terminus modified

full-length

1

144

183

C-terminus

exposed: YES
capacity: HIGH
rate of success: HIGH
Main problems of the HBc: capacity and exposition

VECTOR CLASSES: C-terminus modified

full-length

1
144
183

~100 foreign aa

capacity: HIGH
rate of success: HIGH

exposed: YES
Main problems of the HBc: capacity and exposition

VECTOR CLASSES: C-terminus modified

full-length

1 144 183

~30 foreign aa

exposed: YES capacity: HIGH rate of success: HIGH
Purification of chimeric HBc derivatives
Purification of chimeric HBc derivatives

YIELDS:  
- *Escherichia coli* – 100-150 mg of pure HBc from 1 l of culture
- yeast – 200-300 mg of pure HBc from 1 l of culture
Packaging of HBc particles
Chimeric HBc, which contains immunodominant T helper and CTL epitope

Agarose gel electrophoresis, ethidium bromide staining: proteins were taken in equimolar concentrations

<table>
<thead>
<tr>
<th>Marker</th>
<th>Full-length</th>
<th>C-terminally truncated</th>
<th>Chimeric HBc packed with CpG</th>
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<tbody>
<tr>
<td>1kbp M</td>
<td>HBc183</td>
<td>HBc145</td>
<td>HBc161</td>
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HBc vectors: packaging – magnetic nanoparticles
Vaccine candidates on the HBc basis
Vaccine candidates: HBV

High immunogenicity of a hydrophilic component of the hepatitis B virus preS1 sequence exposed on the surface of three virus-like particle carriers

Dace Skrstina\textsuperscript{a}, Aiste Bulavaite\textsuperscript{b}, Irina Sominskaya\textsuperscript{a}, Larisa Kovalevska\textsuperscript{a}, Velta Ose\textsuperscript{a}, Dace Priede\textsuperscript{a}, Paul Pumpens\textsuperscript{a, b}, Kestutis Sasnauskas\textsuperscript{b}

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\textsuperscript{b} Institute of Biotechnology, Graiciuna 8, LT-02241 Vilnius, Lithuania
Vaccine candidates: HCV
Vaccine candidates: Malaria

Phase I Trial of an Alhydrogel Adjuvanted Hepatitis B Core Virus-Like Particle Containing Epitopes of Plasmodium falciparum Circumsporozoite Protein

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Phase I Testing of a Malaria Vaccine Composed of Hepatitis B Virus Core Particles Expressing Plasmodium falciparum Circumsporozoite Epitopes

Elizabeth H. Nardin1, Giane A. Oliveira1, J. Mauricio Calvo-Callete1, Kristiane Wetzl2, Carolin Maier2, Ashley J. Birkett2, Pramod Sarpatwar3, Michael L. Corado4, George B. Thornton2,3 and Annette Schmidt2

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Conversion of poorly immunogenic malaria repeat sequences into a highly immunogenic vaccine candidate

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doi:10.1186/1471-2172-7-11

A Malaria Vaccine Candidate Based on a Hepatitis B Virus Core Platform

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Vaccine candidates: flu (M2)

Comparative immunogenicity evaluations of influenza A virus M2 peptide as recombinant virus like particle or conjugate vaccines in mice and monkeys.

Fu TM, Grimm KM, Citron MP, Freed DC, Fan J, Keller PM, Shiver JW, Lian X, Joyce JG.
Department of Vaccine Basic Research, Merck Research Laboratories, West Point, PA 19486, United States. tong-ming_fu@merck.com

Improved design and intranasal delivery of an M2e-based human influenza A vaccine.

Department for Molecular Biomedical Research, VIB-Ghent University, FSVM Building, Technologiopark 527, B-9052 Ghent, Zwijnaarde, Belgium.

Universal influenza A vaccine: optimization of M2-based constructs.

DMBR, Ghent University-VIB, FSVM-building, Ghent (Zwijnaarde), Belgium.

A universal influenza A vaccine based on the extracellular domain of the M2 protein

Sabine Neirynck1, Tom Derco1, Xavier Saelens1, Peter Vanhamschoot1,2, Willy Min Jou1,2 & Walter Fiers1

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A "universal" human influenza A vaccine.

Fiers W, De Fillette M, Birkett A, Neirynck S, Min Jou W.
Laboratory Molecular Biology, University Ghent & VIB, Ledeganckstreet 35, 9000 Ghent, Belgium. fiers@dmb.rug.ac.be
Vaccine candidates: flu (M2)

chemical coupling


Influenza A vaccine based on the extracellular domain of M2: weak protection mediated via antibody-dependent NK cell activity.

Jegerlehner A, Schmitz N, Storni T, Bachmann MF.
Cytos Biotechnology, Schlieren, Switzerland.

more complicated fusions


Enhancement of mucosal immune response against the M2eHBc+ antigen in mice with the fusion expression products of LTB and M2eHBc+ through mucosal immunization route.

Zhang GC, Li DX, Zhang HH, Zeng YM, Chen L.
The Key Laboratory of Ministry of Education for Cell Biology and Tumor Cell Engineering, School of Life Sciences, Xiamen University, Xiamen, Fujian, 361005, China.
Follow-up of VLP technologies

8 Artificial Genes for Chimeric Virus-Like Particles
Paul Pompens and Elmars Grens

CRC Press, 2002

9 Construction of Novel Vaccines on the Basis of Virus-Like Particles: Hepatitis B Virus Proteins as Vaccine Carriers
Paul Pompens, Rainer C. Ulrich, Kestutis Sagonaskas, Andris Kazaks, Veta Ose, and Elmars Grens

CRC Press, 2008
VLP: Latvian Biomedical Research and Study Centre
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