Protection induced by early stage of vaccination with influenza virus-like particles

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• **Background**
  
  • **We know:**
  • Influenza vaccine takes about 2 - 4 weeks to develop antibodies to protect against influenza virus infection.
  • Influenza virus-like particle (VLP) is a promising vaccine candidate.
  
  • **We don’t know:**
  • Protection (antibody response) induced before 2 weeks post-vaccination

• **Methods**
  
  • **Vaccine:** VLPs (A/California/04/09, hemagglutinin (HA) and M1
  • Balb/c mice, single immunization (IM, 10 µg of VLPs).
  • For challenge studies, IN (10 LD50) challenge infections started at very early stage of vaccination
  • IgG, IgG subtype, ASC in spleen and bone marrow, lung IgG, lung cytokine, lung virus titer, mouse body weight changes and survival
Gene cloning (HA, M1)

Influenza matrix protein (M1)

M1 (EcoRI / XhoI)

M1 (EcoRI / XhoI)

HA (EcoRI / XhoI)

Influenza hemagglutinin (HA)

Influenza A/California/04/09 M1-pFastBac was transformed into E-coil DH5a and plated on Luria-Bertani (LB) agar plate contained 50ug/ml ampicillin supplemented. Extraction plasmid in single colony and digested with restriction enzymes.
Fig 1. VLPs characterization

A. Silver stained SDS-PAGE
B, C: Western blot
D. Electron microscopy
Fig 2. Virus-specific total IgG antibody responses

Day or month post-vaccination

D0 D1 D2 D3 D4 D7 D10 D14 D21 D28 D35 D42 D80 M4

IgG (OD 450 nm)

0.0
0.1
0.2
0.3
0.4
0.5
0.6
1.0
1.1
1.2
1.3
1.4

IgG Titer
D 0: 100
D 4: 400
D 7: 400
D 14: 3200
D 28: 12800
D 42: 19600
D 80: 19600
M4: 19600

*: P < 0.05, **: P < 0.01
Fig 3. Virus-specific IgG subtype responses
Fig 4. HAI titers at different periods after vaccination

A. HAI titer

B. Neutralizing activity
Fig 5. Lung virus titers and survival post-challenge at day 4 and 7 after vaccination. Protection induced by various periods after vaccination.
Fig 6. IgG antibody responses in the lung
Antibody secreting cell (ASC) responses
Lung inflammatory cytokine responses

A

Lung IgG (450 nm)

B

Spleen IgG (450 nm)

C

Bone marrow IgG (450 nm)

D

Lung IL-6 (pg/ml)
Fig 7. Immune correlates

- **IgG (OD 450 nm)**: \( Y = 14.86 - 10.33X, R = -0.78, P < 0.01 \)
- **IgG2a (OD 450 nm)**: \( Y = 20.63 - 15.88X, R = -0.97, P < 0.001 \)
- **IgG1 (OD 450 nm)**: \( Y = 22.82X, R = -0.68, P < 0.01 \)
- **HAI**: \( Y = 16.52 - 0.12X, R = -0.73, P < 0.01 \)
- **Neutralizing activity**: \( Y = 18.84 - 0.15X, R = -0.94, P < 0.001 \)
<table>
<thead>
<tr>
<th>Time post-vaccination</th>
<th>Immune response parameters</th>
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<tbody>
<tr>
<td></td>
<td>BW (%)</td>
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<tr>
<td>D 0 (Naïve)</td>
<td>14.5</td>
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<tr>
<td>D 1</td>
<td>12.0</td>
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<tr>
<td>D 2</td>
<td>15.4</td>
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<td>D 80</td>
<td>2.0</td>
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<tr>
<td>Month 4</td>
<td>0.7</td>
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Table 1. Immune correlates of protection against pandemic influenza A/California/04/2009
• **Conclusion**

  • VLP vaccination induced significantly higher levels of IgG antibody responses and high HAI titers at day 4 post-vaccination.

  • At day 7, predominant IgG2a antibody responses and viral neutralizing antibodies were induced.

  • Upon challenge on day 4 and 7 post-vaccination, lung IgG antibody responses and recall IgG antibody-secreting cell responses were induced.

  • Lung virus titers decreased significantly at day 7 compared to that at day 4 post-vaccination. The lung virus titer at day 4 post-vaccination also decreased significantly compared to naïve control.

  • This study demonstrates that VLP vaccination provides effective protection in the early stage of vaccination.