Montanide™ adjuvants for stimulation of cellular immune response in FMD vaccines

Sébastien Deville, Juliette Ben Arous, François Bertrand, Laurent Dupuis
W/O and W/O/W adjuvants:

OIL ADJUVANTS ARE A REFERENCE FOR FMD VACCINES IN CATTLE AND SWINE

WATER in OIL

High shear formulation

Viscosity | Stability
---|---
40 mPa.s at 25°C | At 4°C : >12 months At 25°C: ~6 months At 37°C: ~15 days

WATER in OIL IN WATER

Low shear formulation at 30°C

Viscosity | Stability
---|---
25 mPa.s at 25°C | At 4°C : ~12 months At 25°C: ~1 month At 37°C: few days

OIL ADJUVANTS ARE A REFERENCE FOR FMD VACCINES IN CATTLE AND SWINE
Introduction: W/O adjuvants for FMD vaccines

FMD W/O Vaccines for Cattle:
2ml injection IM.
2 shots in the 1st year + 1 shot/year

W/O Cattle trial: Protocol
2 shots protocol, 2ml injection IM.
Duration: 12 month,
Bleeding monthly

Vaccine groups:
- Montanide ISA 50 V2 (W/O)
- AlOH
- Control

References:
W/O/W vaccines for Cattle or Swine
2ml injection IM.
2 shots in the 1st year + 1 shot/year

W/O/W Cattle trial: Protocol
2 shots protocol, 2ml injection IM.
Duration: 80 days
Bleeding day 7, 14, 21, 28, 35, 60, 78

Vaccine groups:
- Montanide ISA 206 VG (W/O/W)
- AlOH+QuilA
- AlOH+Saponine

References:

CROSS-PROTECTION IN FMD VACCINES??

- FMDV: 7 SEROTYPES AND DIVERSE SUBTYPES
- FMDV VACCINES MOSTLY INDUCE A HUMORAL IMMUNE RESPONSE AND ARE NOT CROSS-PROTECTIVE.
- MULTIVALENT VACCINES ARE USED

CD8+ T CELL IMMUNE RESPONSE TO FMDV IS CROSS-REACTIVE (Guzman et al, J. Virology 2010)

FMD VACCINE ADJUVANTS THAT PROMOTE BOTH CELLULAR AND HUMORAL IMMUNE RESPONSES COULD ENHANCE CROSS-PROTECTION IN FMD VACCINES.
Adjuvants to promote cellular immune response: A new Montanide™ range

BASED ON A SPECIFIC LIGHT MINERAL OIL
TO PROMOTE HUMORAL AND CELLULAR RESPONSE:

WATER in OIL

- MONTANIDE ISA 50 V2
- MONTANIDE ISA 70 M VG

WATER in OIL in WATER

- MONTANIDE ISA 206 VG

SPECIFIC FORMULATION
FOR CELLULAR RESPONSE

- MONTANIDE ISA 61 VG (60% w/w)
- MONTANIDE ISA 71 VG (70% w/w)

- MONTANIDE ISA 201 VG (50% w/w)
W/O and W/O/W cellular adjuvants: Galenic studies

EMULSION CHARACTERISTICS ON PLACEBO ANTIGENIC MEDIA

WATER in OIL:
ISA 71 VG / ISA 61 VG
High shear formulation

<table>
<thead>
<tr>
<th>Viscosity</th>
<th>Stability</th>
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<tbody>
<tr>
<td>40 mPa.s</td>
<td>At 4°C: &gt;12 months</td>
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<tr>
<td></td>
<td>At 25°C: ~6 months</td>
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<tr>
<td></td>
<td>At 37°C: ~15 days</td>
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WATER in OIL IN WATER:
ISA 201 VG
Low shear formulation at 30°C

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<td></td>
<td>At 37°C: few days</td>
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</tbody>
</table>
ISA 61 VG induces higher IgG2a titers than ISA 70 VG

WATER in OIL: ISA 61 VG
Animals: OF1 mice 20days
Model antigen: OVA
2 shots protocol, 0.1ml injection SC.
Duration: 90 days,
Bleeding day 14, 28, 42, 56, 90
ELISA IgG1 and IgG2a

Vaccine groups:
- Montanide ISA 70 VG (W/O)
- Montanide ISA 61 VG (W/O)
W/O and W/O/W cellular adjuvants: Model animal studies

ISA 201 VG induces higher IgG2a titers than ISA 206 VG

W/O/W: ISA 201 VG
Animals: OF1 mice 20 days
Model antigen: H1N2
2 shots protocol, 0.1ml injection SC.
Duration: 90 days,
Bleeding day 14, 28, 42, 56, 90
ELISA IgG1 and IgG2a

Vaccine groups:
- Montanide ISA 206 VG (W/O/W)
- Montanide ISA 201 VG (W/O/W)
PROTOCOL:

Animals: 7 to 8 calves/group, 45-75 days old
Antigen: Inactivated coronavirus
2 shots protocol at D0 and D28, 2 ml injection IM in the neck.
Duration: 56 days.
Safety: Assessment of local reactions during trial and at slaughter.
Bleeding D0, 14, 21, 28, 42, 56.
ELISA IgG1 and IgG2

Vaccine groups:
- Antigen + Montanide ISA 70 VG (W/O, 70% w/w)
- Antigen + Montanide ISA 71 VG (W/O, 70% w/w)
- Antigen + Alum
- Antigen / No adjuvant
**SAFETY:**
- No pyrogenic reaction in any group
- No local reaction after primo-vaccination
- Transient reactions observed after revaccination (2cmx2cm)
- Small granuloma observed after dissection of the injection site (slaughter only 28 days after 2nd injection).

<table>
<thead>
<tr>
<th>Vaccine groups</th>
<th>24h</th>
<th>48h</th>
<th>72h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen in saline</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>Alum</td>
<td>2/7</td>
<td>1/7</td>
<td>0/7</td>
</tr>
<tr>
<td>ISA 70 VG</td>
<td>6/8</td>
<td>5/8</td>
<td>1/8</td>
</tr>
<tr>
<td>ISA 71 VG</td>
<td>4/7</td>
<td>2/7</td>
<td>0/7</td>
</tr>
</tbody>
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**Frequency of local reactions after the boost**

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<th>Nb reactions/ Nb animals</th>
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<tbody>
<tr>
<td>Antigen in saline</td>
<td>0/4</td>
</tr>
<tr>
<td>Alum</td>
<td>0/4</td>
</tr>
<tr>
<td>ISA 70 VG</td>
<td>1/6</td>
</tr>
<tr>
<td>ISA 71 VG</td>
<td>3/4</td>
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**Frequency of granuloma at the injection site**

*No pyrogenicity*
*No problematic local reactions*
CASE STUDY: Bovine viral vaccine trial

Efficacy: IgG1 response

- **IgG1 titers (log)**
- **% IgG1 positive animals**
- (log IgG1 titer > 7.6)

ISA 71 VG and ISA 70 VG induce similar IgG1 responses
EFFICACY: IgG2 Cellular response

ISA 71 VG induces a higher IgG2 response than ISA 70 VG and Alum
Conclusions

Cellular response may enhance cross-protection in FMDV vaccines

New Montanide™ range based on a specific mineral oil designed to enhance cellular immune response with:

- safety, IgG1 response
- Specific IgG2 response demonstrated in mice and cattle

- ISA 71VG ; ISA 61 VG ; ISA 201 VG

- Conserved stability
- Easy industrial transposition

W/O VACCINES
BOVINE AND SMALL RUMINANTS
MONTANIDE ISA 61 VG

W/O/W VACCINES
BOVINE AND SWINE
MONTANIDE ISA 201 VG
THANK YOU!