Toxin Derivatives and Other “Danger Signals” as Mucosal Adjuvants

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Toxin Derivatives and Other “Danger Signals” as Mucosal Adjuvants

- Bacterial ADP-ribosylating Enterotoxin (BARE) adjuvants promote the development of both humoral and cell-mediated immune responses in both the systemic and mucosal compartments.
- BAREs can be attenuated sufficiently to reduce or eliminate toxicity while preserving adjuvanticity.
- BAREs can induce mucosal immune responses following mucosal or parenteral immunization.
- The ability of BAREs to induce (mucosal) immune responses is directly related to the ability to elicit TH17 biasing cytokines (IL-1, IL-6, G-CSF).
- BARE-stimulated DCs enhance CD4 clonal expansion and antigen-specific Th1, Th2, Th17, and Treg T-cells.
Adjuvants: Multiple “Styles & Flavors”

- Mineral Salts: aluminum
- Derivatives of bacterial products (MPL, MDP, CpG, BAREs)
- Lipid containing vesicles/emulsions
- Particulates/carriers
- Cytokines
- Synthetic constructs
Where Do Adjuvants Act?

Antigen presentation.
Antigen recognition and APC (or T/B cell) activation.
Co-stimulation.
NOD-like Receptors (bacteria)
- Peptidoglycan, muropeptides, DAP
- Peptidoglycan, muropeptides, MDP
- Bacterial RNA, uric acid crystals, toxins, MDP

RIG-like Helicases (viruses)
- 5'-triphosphate RNA, ssRNA (VSV, parainfluenza virus, influenza virus, JEV)
- Poly (I:C), picornavirus

Surface TLRs and agonists
- Triacyl lipopeptides, Pam3Cys
- Diacyl lipopeptides, Lipoteichoic acids, Zymosan
- LPS, MPL, LPS analogues, Taxol
- Flagellin
- Uropathogenic bacteria, Toxoplasma profilin

Endosome TLRs and agonists
- dsRNA, Poly (I:C)
- ssRNA, Imidazoquinolines (Imiquimod, resiquimod (R848))
- ssRNA, Imidazoquinolines (resiquimod (R848))
- Unmethylated CpG DNA, synthetic oligonucleotides

Scavenger receptors
- Gram positive and Gram negative polyanionic ligands, LDL, apoptotic cells

C-type Lectin Receptors
- Polysaccharides

Triggering Receptors
- Expressed on Myeloid cells

Cross-talk
Bacterial ADP-ribosylating Adjuvants (BAREs)

• Cholera toxin (CT)
• Heat labile toxin from *E. coli* (LT)
E. coli Heat-Labile Enterotoxin (LT)
# Safety Data for LT Dose Escalation

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Volunteers</th>
<th>No. with grade 3-5 Stools</th>
<th>Volume (range in ml)</th>
<th>Onset/Duration</th>
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</thead>
<tbody>
<tr>
<td>WC/BS</td>
<td>5</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WC/BS + 0.5 µg LT</td>
<td>3</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.5 µg LT</td>
<td>3</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WC/BS + 2.5 µg LT</td>
<td>3</td>
<td>1</td>
<td>520</td>
<td>31/26</td>
</tr>
<tr>
<td>2.5 µg LT</td>
<td>3</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WC/BS + 5 µg LT</td>
<td>4</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WC/BS + 10 µg LT</td>
<td>4</td>
<td>2</td>
<td>984-2,200</td>
<td>12/72</td>
</tr>
<tr>
<td>10 µg LT</td>
<td>3</td>
<td>2</td>
<td>242-740</td>
<td>9.5/43</td>
</tr>
<tr>
<td>WC/BS + 15 µg LT</td>
<td>3</td>
<td>1</td>
<td>2,935</td>
<td>12/48</td>
</tr>
<tr>
<td>15 µg LT</td>
<td>3</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WC/BS + 25 µg LT</td>
<td>2</td>
<td>2</td>
<td>2,197-6,372</td>
<td>8/39</td>
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<tr>
<td>25 µg LT</td>
<td>5</td>
<td>1</td>
<td>1,702</td>
<td>8.5/37</td>
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</table>
Can adjuvanticity be separated from enterotoxicity?
Examples of A-Subunit Mutations in LT

Active-Site Mutants

- S63K
- E112K

Protease-Site Mutants

- A72R
- R192G
- L211A

A2-A1 Activation Loop Mutant
Adjuvant Properties of Native LT and LT Mutants

- Promote the development of both humoral (antibody) and cell-mediated immune responses against co-administered antigens in both the systemic and mucosal compartments
- Function as an effective adjuvants when administered mucosally (orally, intranasally, sublingually, rectally), parenterally, and topically (transcutaneously)
- Adjuvant activity is cAMP-dependent
Human Trials with Native LT

- Dukarol (cholera vaccine) oral
- Influenza nasal (Berna)
- CS6 Transcutaneous
- Helicobacter oral
- Influenza Transcutaneous (IS Patch)
- Traveler’s diarrhea (Phase III)
Human Trials with LT(S63K)

- *Mycobacterium tuberculosis* nasal
- HIV nasal

Human Trials with LT(R192G)

- *Helicobacter* oral
- *Campylobacter* oral
- CS6 Transcutaneous
LT, mLT, dmLT

Proposed Mechanisms of BAREs Adjuvanticity

• BAREs mature DCs and induce Th17-biasing cytokine secretion (IL-1, IL-6, G-CSF)

• Adjuvant-stimulated DCs, enhance CD4 clonal expansion and antigen-specific Th1, Th2, Th17, Treg T-cells
Dendritic Cells Are Critical Initiators of BARE-Induced Immunity

Short-term dendritic cell depletion prevents adjuvanticity (oral CT + ovalbumin vaccination)

**Effects on Dendritic Cells**

**Maturation (24h)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CD80 MFI</th>
<th>CD86 MFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT</td>
<td>**</td>
<td>***</td>
</tr>
<tr>
<td>mL</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>dLT</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>E112K</td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>LTB</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>untreated</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Th17 Biasing Cytokines (day 5 culture, pg/ml)**

- **IL-1β**: 300, 300, 300, 300, 300
- **IL-8**: 2500, 2500, 2500, 2500, 2500
- **IL-6**: 2500, 2500, 2500, 2500, 2500
- **G-CSF**: 80, 80, 80, 80, 80

0.1μg LT protein

---

**LT protein**

- LT
- mL
- dLT
- E112K
- LTB
- untreated

**Control**

- untreated

**LTB**

- lt
- mLT
- dLT
- E112K
- LTB
- untreated

**E112K**

- E112K
- LTB
- untreated

**LTB**

- LTB
- untreated
DC/T-Cell Interaction

LT-treated/washed

5 days

T-cell Proliferation & Cytokine secretion

0.1µg DC pre-treatment

LT
mLT
dmLT
E112K
LTB
untx

CD4 T-cell CFSE

400
1000
800
600
400
1000
800
600

CD4 T-cell CFSE

IL-17

LT
mLT
dmLT
E112K
LTB
untreated
1.) dendritic cell recruitment

2.) dendritic cell activation

3.) Expansion of CD4: Th1, Th2, Th17
Contributions of IL-17 to Adjuvant-Enhanced Immunity

• Upregulate plg receptor on basolateral surface of epithelial cells
• Augment chemokine induction by epithelial cells
• Increase recruitment of neutrophils, monocytes and macrophages
• Facilitate class switching to IgA
• Increase germinal centers
• Induction of antimicrobial peptides
adjuvants:
- bacterial enterotoxins (LT, CT)
- TLR-agonists (MPL, CpG, flagellin)
- non-TLR immunostimulants (immune activators, soluble factors)

particulate carriers:
polymer and lipid based particles
- lectin
- β-glucan

<table>
<thead>
<tr>
<th>Adjuvant</th>
<th>Receptor</th>
<th>Cytokines Produced by APC</th>
<th>T helper response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptidoglycan</td>
<td>TLR2</td>
<td>IL-23, IL-1β</td>
<td>IL-17</td>
</tr>
<tr>
<td>Lipoteichoic Acid</td>
<td>TLR2</td>
<td>IL-23</td>
<td></td>
</tr>
<tr>
<td>LPS</td>
<td>TLR4</td>
<td>IL-23, IL-6, IL-1β, IL-12</td>
<td>IL-17, IFNγ</td>
</tr>
<tr>
<td>Resimiquod (R848)</td>
<td>TLR7/8</td>
<td>IL-23, IL-6, IL-1β</td>
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</tr>
<tr>
<td>Pam2C</td>
<td>TLR2/6</td>
<td>IL-23</td>
<td></td>
</tr>
<tr>
<td>Pam3C</td>
<td>TLR1/2</td>
<td>IL-23</td>
<td></td>
</tr>
<tr>
<td>LPS+R848</td>
<td>TLR4/7/8</td>
<td>IL-12, IL-23</td>
<td>IL-17, IFNγ</td>
</tr>
<tr>
<td>MDP+Pam2C or Pam3C</td>
<td>NOD2, TLR1/2/6</td>
<td>IL-23</td>
<td>IL-17</td>
</tr>
<tr>
<td>B-glucan</td>
<td>Dectin 1</td>
<td>IL-23, IL-10</td>
<td>IL-17</td>
</tr>
<tr>
<td>CpG</td>
<td>TLR9</td>
<td>IL-12, IFNγ</td>
<td></td>
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</tbody>
</table>

Th17 Cells Involved in Activating Innate and Adaptive Immune Responses Important for Protection Against Mucosal Infections


<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Vaccine</th>
<th>Organ</th>
<th>Mechanism/reference</th>
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<tbody>
<tr>
<td><em>M. tuberculosis</em></td>
<td>Peptide vaccine</td>
<td>Lung</td>
<td>Recruitment of CD4 Th1 cells [47]</td>
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<tr>
<td><em>M. tuberculosis</em></td>
<td>Subunit vaccine</td>
<td>Lung</td>
<td>[2]</td>
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<tr>
<td><em>M. tuberculosis</em></td>
<td>Viral vectors expressing protective antigen</td>
<td>Lung</td>
<td>[38]</td>
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<tr>
<td><em>M. tuberculosis</em></td>
<td>Primary BCG vaccination followed by booster</td>
<td>Lung</td>
<td>[102]</td>
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<tr>
<td><em>M. tuberculosis</em></td>
<td>with viral vectors expressing protective antigens</td>
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<tr>
<td><em>M. tuberculosis</em></td>
<td>Primary BCG vaccination followed by booster</td>
<td>Lung</td>
<td>[87]</td>
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<tr>
<td><em>M. tuberculosis</em></td>
<td>with DNA vaccine encoding protective antigens</td>
<td>Lung</td>
<td>[108]</td>
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<tr>
<td><em>M. tuberculosis</em></td>
<td>DNA vaccine expressing protective antigen</td>
<td>Lung</td>
<td></td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>Pneumococcal whole-cell antigen</td>
<td>Lung</td>
<td>[11]</td>
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<tr>
<td><em>S. pneumoniae</em></td>
<td>Cell wall polysaccharides</td>
<td>Lung</td>
<td>Monocyte, macrophage, and neutrophil</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>recruitment and phagocytic killing [63, 64]</td>
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<tr>
<td><em>S. pneumoniae</em></td>
<td>Live organism</td>
<td>Lung</td>
<td>Monocyte, macrophage, and neutrophil</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>recruitment [114]</td>
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<td><em>B. pertussis</em></td>
<td>Whole cell vaccine</td>
<td>Lung</td>
<td>Neutrophil recruitment and enhanced</td>
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<td></td>
<td>phagocytic killing [39]</td>
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<tr>
<td><em>H. pylori</em></td>
<td><em>H. pylori</em> lysate and recombinant urease</td>
<td>Gut</td>
<td>Recruitment of neutrophils, monocytes and</td>
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<td></td>
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<td>macrophages and enhanced killing activity</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>[21, 100]</td>
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<tr>
<td><em>P. aeruginosa</em></td>
<td>Live organism</td>
<td>Lung</td>
<td>Recruitment of neutrophils and increased</td>
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<td>bacterial clearance [86]</td>
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<td><em>Rhesus rotavirus</em></td>
<td>Recombinant antigen</td>
<td>Intestine</td>
<td>[72, 95, 99]</td>
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<td><em>Influenza virus</em></td>
<td>DNA vaccine expressing protective antigen and IL-23</td>
<td>Lung</td>
<td>[106, 107]</td>
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<td><em>Eimeria acervulina</em></td>
<td>Recombinant antigen and IL-17</td>
<td>Intestine</td>
<td>[22]</td>
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</tbody>
</table>
Protection Following SL and Buccal Immunization With Whole-Cell Pneumococcal Vaccine

Adapted from Lu et al. Clin Vaccine Immunol. 17:1005-1012, 2010
Protection Following Transcutaneous Immunization With Whole-Cell Pneumococcal Vaccine

Adapted from Lu et al. Clin Vaccine Immunol. 17:1005-1012, 2010
BAREs Can Enable Mucosal Immune Response Following Parenteral Immunization

ID immunization, 3x weekly, 5 µg TT + 1 µg dmLT
Intradermal Vaccination Directs Fecal Anti-Adhesin Response

p < 0.001

With permission of Stephen J. Savarino, NMRC, US Army Medical Research and Materiel Command
dmLT Functions as Both an Adjuvant and an Immunogen for Induction of Fecal IgA Following Oral Immunization

n.d. – none detected
n.a. – not applicable
Groups of 10 BALB/c mice each were immunized at day 0 and 21 and sacrificed at day 35. Immunizations consisted of IPV (.01, 0.1, 1.0, 10 DU PV-1) alone or admixed with 1.0 μg of dmLT.
Summary and Current Status of dmLT

• Safe, effective adjuvant for oral, intradermal, subcutaneous, intramuscular, transcutaneous and sublingual delivery

• Induces systemic and mucosal IgG and IgA when delivered mucosally or parenterally

• Effective adjuvant AND antigen for ETEC vaccines

• Completed NIAID sponsored Phase I clinical trial (clinicaltrials.gov identifier NCT01147445)

• Ongoing Phase I clinical trial of dmLT as one component of an oral ETEC vaccine (EudrCT No. 2011-003228-11)

• Ongoing Phase I clinical trial of dmLT as one component of an oral ETEC vaccine (Johns Hopkins University)
Summary and Current Status of mLT

- Ongoing Phase I clinical trial of mLT as one component of a transcutaneous ETEC vaccine (clinicaltrials.gov identifier NCT01382095)

- Ongoing Phase I clinical trial of mLT as one component of a transcutaneous or intradermal ETEC vaccine (clinicaltrials.gov identifier NCT01644565) - recruiting
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