Review of Immune Stimulants to Support Bovine Health

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History

• 1890’s: Dr. William Coley recognized that bacterial infection sometimes caused tumors to regress in human patients
  – Administered killed Gram positive + Gram negative bacteria (“Coley’s toxin”) into tumors to induce tumor regression and improve patient survival

Definition/terminology

• Immunostimulant: therapeutic that nonspecifically stimulates the immune response for beneficial purposes
  – aka “immunomodulator”
  – aka “biological response modifier”

• Adjuvant: compound added to vaccine to improve vaccine-induced immunity
  – Many adjuvants can also be used as immunostimulants
Indications for immunostimulants

- Situations of unusual stress leading to
  - immune suppression and/or
  - excessive infectious agent challenge
    • e.g.: transport, mixing
- At-risk neonates
  - due to immature/naïve immunity
- To decrease metabolic cost of immunity
  - nonspecific “priming” of immune response may make later specific stimulation less metabolically “costly”

From Blecha, 2001

Classical immunostimulants

- Microbial products
  - bacteria:
    • e.g., inactivated P. acnes, lysed S. aureus, components of LPS, mycobacterial cell walls
  - viruses:
    • parapoxviruses (Baypamun)
  - fungi:
    • β-glucan, mannan oligosaccharides
- Cytokines
  - interferons, IL-2, colony stimulating factors, proinflammatory cytokines (IL-1β, IL-6, TNF-α)

Classical immunostimulants

- Pharmaceuticals
  - levamisole, isoprinosine, macrolides
- Nutraceuticals (“functional foods”)
  - vitamins and minerals
  - isoflavones, conjugated linoleic acid
- Traditional medicinal plants or their products
  - aloe vera (acemannan)
  - ginseng
Some examples of classical immunostimulant use: cattle

- Prophylactic administration of P. acnes enhanced clearance of M. haemolytica Al-Izzi and Maxie, 1982
- Interferon-α diminished effects of experimental BHV-1 challenge Cummins et al., 1993
- Baypamun N decreased respiratory disease in transported veal calves Ziebell et al., 1997
- β-glucan + ascorbic acid did not clearly improve health of dairy calves post transport Eicher et al., 2010

- In spite of some supportive evidence for various classical immunostimulants, none have been widely adopted
- Possible reasons
  -- Unfavorable cost-benefit ratio
  -- Management-related constraints (e.g. required timing of administration)
  -- Insufficient proof of efficacy
- Little published research to allow evidence-based recommendations

- Historically, the mechanism of action of many immunostimulants was poorly characterized
- Recently: many immunostimulants recognized to include PAMPs or PAMP-like molecules
  -- Presumably activate immunity through stimulation of pathogen recognition receptors (PRR) on sentinel cells
Sites where cellular pathogen recognition receptors (PRR) can bind to PAMPs or DAMPs

Sentinel cell

- On the cell surface
  - TLR-1, TLR-2, TLR-3, TLR-4, TLR-5, TLR-6, TLR-7, TLR-8, TLR-9

- Within endosomes inside of cell
  - CLR

- Within the cytoplasm
  - MDAR, RIG-I, NLR

### Innate Immune Recognition and Cellular Distribution by Mammalian Toll-like Receptors

<table>
<thead>
<tr>
<th>Toll-like receptor</th>
<th>Signal</th>
<th>Cellular distribution</th>
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<tbody>
<tr>
<td>TLR-1/TLR-2 heterodimer</td>
<td>Lipopolysaccharide, flagellin</td>
<td>Macrophages, dendritic cells, mast cells, eosinophils, basophils</td>
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<tr>
<td>TLR-3</td>
<td>Double-stranded RNA (virus)</td>
<td>NK cells</td>
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<tr>
<td>TLR-4 + MD-2 + CD14</td>
<td>LPS (Gram-negative bacteria), lipoteichoic acids (Gram-positive bacteria)</td>
<td>Macrophages, dendritic cells, mast cells, eosinophils, basophils</td>
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<tr>
<td>TLR-5</td>
<td>Flagellin (bacteria)</td>
<td>Dendritic cells</td>
</tr>
<tr>
<td>TLR-7</td>
<td>Single-stranded RNA (virus)</td>
<td>Dendritic cells, T cells, eosinophils, B cells</td>
</tr>
<tr>
<td>TLR-8</td>
<td>Single-stranded RNA (virus)</td>
<td>NK cells</td>
</tr>
<tr>
<td>TLR-9</td>
<td>Unmethylated CpG DNA (bacteria, herpesvirus)</td>
<td>Dendritic cells, eosinophils, B cells, basophils</td>
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- Binding of PRR by a PAMP initiates a signal transduction sequence in the cell
- This will cause the cell to produce cytokines that will in turn activate the inflammatory/immune response
- The mixture of cytokines produced will determine the kind of immune response activated
TLR ligands as immunomodulators

- Synthetic molecules that bind to TLR are being developed for use in treating infectious diseases, autoimmune diseases, and cancer
- Agonists: activate inflammation/immunity
  - Help resolve infections or treat cancer
- Antagonists: suppress inflammation
  - Counteracts excessive inflammation
  • sepsis, autoimmunity

Hennessy et al., 2010

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TLR agonists and antagonists for treatment of infection in humans (in development)

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<tr>
<th>Compound</th>
<th>Class</th>
<th>Indication</th>
<th>Status</th>
<th>Target</th>
<th>Mode</th>
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Hennessy et al., Nat Rev Drug Discov 9:293, 2010

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Immunostimulants: newer models

- CpG DNA
  - small segments of DNA containing cytosine next to guanine on the same strand (as opposed to base-paired)
  - very common in some bacteria and viruses
    • in bacteria are usually unmethylated (in contrast to mammalian DNA)
- Unmethylated CpG DNA are a PAMP
  - Recognized by TLR9 and other intracellular PRR
• In general, CpG DNA stimulates strong TH1 responses
  – especially good for intracellular infections
    • viruses, certain bacteria (mycobacteria, *Salmonella*)
• Can even downregulate established TH2 responses
  – Potentially good for treating autoimmunity or allergies

CpG DNA in food animals: a few examples
• Pre-treatment of lambs with CpG DNA decreased viral shedding following BHV-1 or PI3V challenge
  Nichani et al., 2006
• Pre-treatment of chickens with CpG DNA improved survival following *E. coli* challenge  Gomis et al., 2003
• Addition of CpG DNA to a subunit BHV-1 vaccine improved humoral and cell-mediated immunity in calves  Ioannou et al., 2002

• HOWEVER: CpG DNA don’t ALWAYS work as expected:
  – CpG DNA did not improve resistance of swine to influenza challenge  Mutwiri, 2012
  – CpG DNA reduced survival of mice in a model of *Candida albicans* infection  Ito et al., 2005
Possible mechanisms of immune enhancement mediated by CpG DNA:

- enhanced maturation and differentiation of dendritic cells
- improved activation of B cells
- suppression of B cell apoptosis

reviewed in Mutwiri, 2012

CpG DNA: challenges

- Problems with CpG DNA:
  - When administered “naked” or “free”: too rapidly cleared or degraded in vivo
  - Unfavorable biodistribution
  - Poor cellular uptake (required for function)

*negative charge of DNA repelled by negative charge of cell surface*

Wilson et al., 2009

macrophage
• One solution:
  – Complex CpG DNA with **cationic lipid**
    • cationic group on one end of long lipid chain
    • may form liposomes or lipoplexes
      – liposomes: single or multiple concentric spheres of lipid surrounding aqueous interior
      – lipoplexes: heterogeneous complexes
Uptake of CpG DNA by macrophages (CD11b+) or dendritic cells (CD11c+) is enhanced when they are complexed to cationic lipids

Wilson et al., 2009

- Cationic lipids alone (without CpG DNA) can also activate immune responses
  - Induce cytokine expression by dendritic cells
  - Induce costimulatory molecule expression by dendritic cells
    - Necessary for proper activation of T cells

- To recap:
  - CpG DNA and cationic lipids both stimulate immune responses through various pathways
  - The two combined have the potential to induce more broad immunomodulation
Caveats/cautions

- CpG DNA do not always activate immunity as expected
- Cationic lipids can sometimes induce apoptosis (programmed cell death)
  - If immune cells undergo inappropriate apoptosis: immune dysfunction?
- Could co-administration with some vaccine adjuvants induce excessive and harmful inflammation?
  - OR: could co-administration improve effects of some vaccines?

Newer model immunostimulants, labeled for use in cattle

- Zelnate™ (Bayer): for aid in the treatment of bovine respiratory disease due to M. haemolytica...at the time of a stressful event
- Imrestor™ (Elanco): for reduction of clinical mastitis in the first 30 days of lactation in dairy cows and heifers

- Zelnate™: CpG DNA (bacterial plasmid) + liposome carrier
- Imrestor™: bovine granulocyte colony stimulating factor (G-CSF), pegylated ("pegbovigrastim")