Innate defense mechanisms and passive immunity

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Effectors of INNATE IMMUNITY during infection

- Physical/chemical barriers
- Neutrophils, macrophages, dendritic cells, NK cells
- Secretion of cytokines, chemokines, antimicrobial peptides incl. host defense peptides
- Complement and coagulation system

http://iahealth.net/inflammation/
## TRAINED INNATE IMMUNITY

Table 1: Overview of innate immune memory mechanisms described for various types of innate immune cells.

<table>
<thead>
<tr>
<th>Innate immune cell type</th>
<th>Primary challenge</th>
<th>Type of memory</th>
<th>Pathway involved</th>
<th>Mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocytes and macrophages</td>
<td>LPS</td>
<td>Tolerance/trained immunity</td>
<td>TLR4/MAPK-dependent, ATF7-dependent</td>
<td>Epigenetic changes: latent enhancers (H3K4me1), other modifications (H3K4me3, H2K27me, H3K9me2)</td>
<td>Foster et al. (50), Ostuni et al. (75), Yoshida et al. (80)</td>
</tr>
<tr>
<td>Monocytes and macrophages</td>
<td>β-glucan, <em>Candida</em> infection, BCG vaccination</td>
<td>Trained immunity</td>
<td>Dectin-1/Raf1/Akt-dependent, STAT1-dependent, NOD2-dependent</td>
<td>Epigenetic changes: (H3K4me1, H3K4me3, H2K27Ac, H3K9me2), metabolic rewiring</td>
<td>Quintin et al. (26), Saeed et al. (51), Cheng et al. (79), Yoshida et al. (80)</td>
</tr>
<tr>
<td>NK cells</td>
<td>Hapten-induced influenza A, vaccinia virus, HIV-1 infection</td>
<td>Antigen-specific</td>
<td>Not described</td>
<td>CXCR6-dependent, NKG2D-dependent</td>
<td>O’Leary et al. (30), Paust et al. (57), Gillard et al. (58), Reeves et al. (64)</td>
</tr>
<tr>
<td>NK cells</td>
<td>CMV infection</td>
<td>Antigen-dependent</td>
<td>Atg3-mediated mitophagy</td>
<td>BNIP3/BNIP3L-dependent</td>
<td>Sun et al. (31), O’Sullivan et al. (61)</td>
</tr>
<tr>
<td>NK cells</td>
<td>CMV infection</td>
<td>Trained immunity</td>
<td>Stable down-regulation of adaptors and transcription factors (e.g., Syk, PLZF)</td>
<td>Epigenetic modification of gene promoters DNA methylation</td>
<td>Lee et al. (65), Schlums et al. (34)</td>
</tr>
</tbody>
</table>
TRAINED INNATE IMMUNITY

Hamon & Quintin, 2016, Sem. Immunol. 28
HOST DEFENSE PEPTIDES (HDPs)

- Invading bacteria (1)
- Recruitment of neutrophils and monocytes (2)
- Suppression of proinflammatory cyto/chemokines & enhancement of anti-inflammatory mediators (3)
- Induction of macrophages and dendritic cell diff. and activation (4)
- Modulation of adaptive immunity (5)
- Regulation of autophagy and neutrophil extracellular traps (NETs) (6)

CYTOKINES: PEGylated G-CSF*

*NGranulocyte colony-stimulating factor

Natalello et al., 2012, PLoS ONE

Met-G-CSF-Gln135-PEG

Met-G-CSF-Met1-PEG

Natalello et al., 2012, PLoS ONE
G-CSF example - bovine mastitis

Table 2—Incidence of clinical mastitis in cattle treated with saline solution or various doses of PEG–bG-CSF.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. (%) of cattle with clinical mastitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline solution (n = 53)</td>
<td>18 (34)</td>
</tr>
<tr>
<td>5 µg/kg (n = 51)</td>
<td>10 (19.6)</td>
</tr>
<tr>
<td>10 µg/kg (n = 54)</td>
<td>9 (16.7)*</td>
</tr>
<tr>
<td>20 µg/kg (n = 53)</td>
<td>5 (9.4)†</td>
</tr>
</tbody>
</table>

*; †Value differs significantly (*P = 0.044; †P = 0.004) from the value for saline solution.

MATERNALLY TRANSFERRED ANTIBODIES
INNATE (temporary) PASSIVE IMMUNITY

Newborn and lactating stages
LACTOGENIC IMMUNITY: TRANSLATION OF MATERNAL GUT IMMUNITY TO MILK/COLOSTRUM IMMUNOGLOBULINS

Langel et al. 2016, Virus Res. 226
MATERNALLY TRANSFERRED ANTIBODIES
INNATE (temporary) PASSIVE IMMUNITY

- Polyclonal and polyspecific, representing maternal immunoglobulin repertoire
- IgG type in most animals with transfer of colostral immunoglobulin in the newborn
- Of utmost importance for survival and health by supplying first systemic immunoglobulins and lactogenic (intestinal) immunity

PrabhuDas et al. 2015, Nature Immunol. 16
TOOLS FOR HANDLING INFECTIOUS DISEASES OF LIVESTOCK

Non-VACCINE IMMUNE METHODS

VACCINES

MANAGEMENT

ANTIBIOTICS
WHICH are the DEMANDS to ALTERNATIVE PRODUCTS?

- Broadly efficient against many different microbial pathogens
- Low cost and ease of use
- Low level of adverse effect, including uncontrollable effects on the immune system
- No risk of induction of resistance
- Immediate effect
- Minimal impact animal product quality
- Minimal negative impact on consumer perceptions
PASSIVE IMMUNITY: IMMUNOGLOBULIN EFFECTORS

Antigen-binding site

Papain cleavage sites

Antigen-binding site

Fab

CL

VH

VN

VH

VH

CH1

CH1

CL

C = constant domain
V = variable domain
H, L = heavy, light chains

Figure 5-21a
Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W.H. Freeman and Company
<table>
<thead>
<tr>
<th>Product type</th>
<th>Animal</th>
<th>Disease prevention/targeted pathogens</th>
<th>Immunoglobulin type/origin</th>
<th>Administration (Oral/parenteral)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> specific antibodies</td>
<td>Calves</td>
<td>Scour</td>
<td>Bovine colostrum IgG/IgY</td>
<td>Oral</td>
</tr>
<tr>
<td>Antibacterial bovine serum antibodies</td>
<td>Cattle</td>
<td><em>Arcanobacterium</em> pyogenes</td>
<td>Bovine serum</td>
<td>Parenteral</td>
</tr>
<tr>
<td></td>
<td>Calves</td>
<td><em>E. coli</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td><em>Mannheimia</em> haemolytica</td>
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<tr>
<td></td>
<td></td>
<td><em>Pasteurella multocida</em></td>
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<tr>
<td></td>
<td></td>
<td><em>Salmonella</em></td>
<td></td>
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<td></td>
<td></td>
<td><em>Typhimurium</em></td>
<td></td>
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<tr>
<td><em>Clostridial</em> antitoxins</td>
<td>Cattle</td>
<td><em>Clostridium perfringens</em></td>
<td>Equine Ig</td>
<td>Parenteral (sc and iv)</td>
</tr>
<tr>
<td></td>
<td>Calves</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Goat</td>
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<tr>
<td></td>
<td>Sheep</td>
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<tr>
<td></td>
<td>Swine</td>
<td></td>
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<tr>
<td></td>
<td>Horses</td>
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</tr>
<tr>
<td>Tetanus Antitoxin</td>
<td>Horses</td>
<td>Tetanus</td>
<td>Equine serum</td>
<td>Parenteral</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-West Nile Virus Antibodies</td>
<td>Horses</td>
<td>West Nile Virus</td>
<td>Equine Ig</td>
<td>Parenteral</td>
</tr>
<tr>
<td>Anti-endotoxin antibodies</td>
<td>Horses</td>
<td>Septicaemia</td>
<td>Equine plasma from hyper-immune horses</td>
<td>Parenteral</td>
</tr>
<tr>
<td>Antibacterial plasma antibodies</td>
<td>Horses</td>
<td><em>Rhodococcus equi</em></td>
<td>Equine plasma from hyper-immune horses</td>
<td>Parenteral</td>
</tr>
<tr>
<td>Equine plasma</td>
<td>Horses</td>
<td>Failure of Passive Transfer</td>
<td>Equine plasma</td>
<td>Both</td>
</tr>
</tbody>
</table>

WIDENING THE SCOPE: WIDESPREAD DISEASES NOT ADRESSED BY VACCINATION OR PASSIVE IMMUNIZATION

• Enteric infectious production diseases commonly treated with antibiotics (major source of veterinary antibiotics usage)
  Post-weaning diarrhea (PWD)(pigs)
  Diarrhea in newborn and young calves
  Rainbow trout fry syndrome: *Flavobacterium psychrophilum*

• Other enteric infectious production diseases (viral or unknown origin)
  Porcine epidemic diarrhea (PED)
  New neonatal porcine diarrhea (NNPD)
  Mink diarrhea

• Zoonoses
  Campylobacter infection in broilers
• Cheap and renewable source of immunoglobulins
  WHEY, BLOOD PLASMA

• Cheap and efficient methods for purification of active immunoglobulins

• Cheap and efficient methods for formulating immunoglobulin for easy oral administration and gut stability
• Cheap and renewable source of immunoglobulins: **WHEY, BLOOD PLASMA**

**Blood plasma**: little used, low value byproduct from slaughterhouses (cattle, pig, poultry, fish; approx. 7% of carcass weight; 2-3 L/pig)

Immunoglobulin content: approx. 10 g/L

**Whey**: little used, low value byproduct from cheese production (mostly cow’s milk)

Immunoglobulin content: approx. 0.7 g/L
• Cheap and renewable source of immunoglobulins
  WHEY, BLOOD PLASMA

• Cheap and efficient method for purification of active immunoglobulins

Expanded bed adsorption (EBA)

• Cheap and efficient methods for formulating immunoglobulin for easy oral administration and gut stability
Efficient method for purification of active immunoglobulins: Expanded bed adsorption (EBA)

Pig IgG purified from 110 liters of pooled pig plasma by EBA

Hedegaard et al., 2016, PLOS ONE 11(1): e0147373. doi:10.1371/journal.pone.0147373
UpFront EBA Technology

Crosslinked Agarose – tungsten carbide conglomerate
Density 2.5-3.5 g/ml
Size range 20-200 micrometer
Stable in hot 1 M NaOH

• Self-creating size gradient stabilise expanded bed
• High density allow high flow and stabilise expanded bed
WHEY: cow herd experiment

35,000 L (35 m³) whey applied in one cycle; yield was around 15,000 g (approx. 60% of theoretical yield)

Heegaard, Larsen, Lihme and Bisgaard-Frantzen, unpublished
WHEY: cow herd experiment

DESIGN: 20 calves were given IgG supplement within first 24 hours (200 g/calf) and then twice daily, each day for 30 days (2x4 g/calf/day = 240 g/calf)
20 control calves did not get any supplement
Final IgG concentrations

Treatment group, 26: CTRL, 28: +IgG product

P=0.0013

Weight gain

NOTE: Groups were meant to be staggered, i.e. calf no. 1 in group A, calf no. 2 in group B, calf no. 3 in A, etc., however, group A (+IgG) consisted of calves 1-20 and group B (CTRL) of calves 21-40

Pneumonia in IgG group!
**CAMPYLOBACTER:** Passive immunization of chickens against *C. jejuni* challenge using goose serum immunoglobulins

Heegaard, Bahrndorff, Madsen, Vigre, Hoorfar and Hald, unpublished
Group 1: Chickens are inoculated orally with 100 mg immunoglobulin and *C. jejuni* (SC181, 10exp4 CFU).

After 2 hours, immunoglobulin inoculation is repeated

N=15 in each group

Group 4: Chickens are inoculated orally with 100 mg immunoglobulin* and *C. jejuni* (SC181, 10exp4 CFU).

After 2 hours, immunoglobulin* inoculation is repeated

*stabilized

Group 8: Chickens are inoculated orally with 100 mg immunoglobulin** and *C. jejuni* (SC181, 10exp4 CFU).

After 2 hours, immunoglobulin** inoculation is repeated

**placebo (denatured bovine IgG)
Cecal CFU *C. jejuni* (inoculation strain)

Passive immunization, 2x100 mg
oral goose immunoglobulin

Mean of group compared to mean of CTRL group;

***: <0.001; **: 0.001 - 0.01; *: 0.01-0.05

Day 7 after inoculation
Fecal CFU *C. jejuni* (inoculation strain)

Passive immunization, 2x100 mg oral goose immunoglobulin

Mean of group compared to mean of CTRL group:
- **:** < 0.001
- ***:** 0.001 - 0.01
- *:** 0.01 - 0.05

Log CFU, Fecal, day 7

Log CFU, Fecal, day 4

Non-stabilized (1)  Stabilized (4)  Control (8)
Pigs, postweaning diarrhea

Hedegaard, C.J., Session 4 presentation and poster
SUMMARY

Can products for creating passive immunity (antibodies) be used as alternatives to antibiotics for treating/preventing the most prominent/antibiotics demanding infection-related diseases in modern animal production?
PASSIVE IMMUNIZATION AS AN INTERVENTION TOOL

- Instantaneous, short-lived effect, not dependent on immune system in host
- Maternal antibodies do not interfere
- The risk of creating resistance is expected to be minimal
- Does not interfere with serology as antibodies remain in the gut and are not transferred to circulation (with some exceptions)
- Relevant antibodies are readily available from blood, milk, whey, eggs, etc
- Cost effective technologies for purifying antibodies are in place
WHICH DISEASE TARGETS?

- Multifactorial infectious diseases and infectious diseases with unknown etiology

- Infectious diseases in very young animals or in other circumstances where the immune system is unresponsive to vaccination

- Infectious diseases for which no vaccines are available or for other reasons not applicable (high cost; not easy to use)

- Infectious disease for which efficient immunity is hard to achieve

- Well-known but infrequent infectious diseases
REMAINING QUESTIONS and CHALLENGES

• Formulation issues for ease of administration and optimal gut stability

• Securing absence of unwanted agents, especially viruses when immunoglobulins are sourced from blood plasma

• Dosing optimization, - for how long do antibodies work in the gut (stabilized/non-stabilized)?

• Are antibodies efficient against establishment of colonization and/or are they able to remove existing colonization?

• Can antibodies provided in the feed protect against non-enteric infections?
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- Pig plasma Ig for post weaning diarrhoea
- Immunoglobulin for fish diseases
Danish AgriFish Agency: Whey Ig for calves (diarrhoea)
Danish Strategic Research Council: Ig from chicken blood (Campylobacter)