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Trained Immunomodulation in Food Animals

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Abstract

Immunomodulation is a potential technique for increasing disease resistance in food animals without using antibiotics. Innate memory is a rapidly growing field of immunomodulation in which innate immunity cells undergo epigenetic changes such as chromatin remodelling and metabolic reprogramming in response to a priming event, resulting in increased or decreased reactivity to secondary stimuli. Over the last decade, significant progress has been made in our understanding of innate training in humans and rodent models, and methods are being developed to precisely target or modify innate memory. The notion of increasing the innate immune system in veterinary species is not new; nevertheless, there are few research that have actively studied innate training as stated in human literature. The development of tailored techniques to activate innate training in food animals with the practical objective of improving the capacity to control illness without using antibiotics is a topic that warrants consideration. The evidence for innate immunomodulation and memory key ideas in feeding animals is summarized in this study.

Keywords: Immunomodulation, innate immunity, food animals

Introduction

Immunomodulation depicts changes occuring in the immune system as a result of exposure to a chemical or molecule (i.e., an agonist) that either stimulates or inhibits the immunological response. Innate memory is a rapidly emerging field of immunomodulation in which innate immunity cells undergo epigenetic modifications such as chromatin re-modeling and metabolic reprogramming in response to a priming event, resulting in increased or decreased reactivity to secondary stimuli (tolerance or training). When cells are educated to respond to subsequent stimulation or exposure, they produce more effector molecules, such as pro-inflammatory cytokines. Because food animals have such a limited lifespan, improvements in innate immunity early in infancy may provide protection against disease for the rest of the animal's life. The primary goal of this review is to summarise the reported evidences for innate immunomodulation and memory important concepts from the literature in cattle, sheep, goats, swine, and poultry, including mechanisms and evidence for innate training in various cell types, as well as discuss the benefits and drawbacks of engaging innate training for disease resistance enhancement.

Mechanisms and Cells in Trained Immunity

Epigenetic reprogramming of innate immune cells, has been observed predominantly in monocytes, is the basis of trained immunity. On secondary stimulation, epigenetic alterations cause turnaround in gene expression and, as a result, protein synthesis. The fundamental mechanisms of epigenetic reprogramming related with trained immunity include chromatin alterations and changes in DNA accessibility. Increased methylation of the latent enhancer histone H3 at K4 (H3K4me1), decreased methylation of the repressor histone marks such as histone H3 at K9 (H3K9me3), and the most informative histone marker, increased acetylation at the poised/active enhancer mark (H3K27ac), are all linked to a trained phenotype (Novakovic *et al.*, 2016). The immune response is regulated by posttranscriptional regulatory mechanisms ex. microRNA (miRNA) modification of mRNA levels. Changes in metabolic status have also been seen in trained cells, which are most likely the consequence of epigenetic reprogramming, which primes cells to react to secondary stimulation. The metabolic state is crucial for quick release of intermediate substrates like nucleic and amino acids, which are required for the creation of effector molecules (O'Neill *et al.,* 2016). Monocyte-derived macrophages and monocytes, as well as NK cells, have well-documented innate training and tolerance. Dendritic cells, non-immune cells including mesenchymal and epithelial stem cells, and intestinal stromal cells have all been found to have innate training.

Immunomodulatory Approaches in Food Animals:

Cattle

There have only been a few studies of inherent training in cattle, as detailed by (Netea *et al.* 2014).

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- In one study, immunisation of 3 to 6 month old calves with heatkilled *M. bovis* increased the ability macrophages to phagocytose and kill cells of *M. bovis* in vitro. Such impact sustaining up to 6 months after immunisation and was independent of humoral or cellular adaptive immune responses (Juste *et al.*, 2016).
- The use of an ultrasonicated lysate of *Corynebacterium cutis* to immunise cattle showed a favourable effect on morbidity and mortality in three age groups of animals. Oral immunisation of calves with *Salmonella enteritidis serovar Typhimurium (S. Typhimurium)* attenuated, live auxotrophic mutants resulted in homologous and heterologous protection against *S. Typhimurium* and *S. Dublin* in a number of experiments (Wray *et al.*, 1977).
- Several new commercial medicines have been developed to improve the innate immune response of cattle during stressful situations. The commercial product Zelnate™, a DNA-based immunostimulant, has been shown to lower lung pathology in calves experimentally challenged with *M. haemolytica*, as well as considerably reduces mortality in high-risk animals following feedlot placement (Woolums *et al.* 2019).
- Another immunomodulatory product, Amplimune™, mycobacterial cell wall fraction from non-pathogenic *Mycobacterium phlei*, can significantly reduce the incidence and severity of K99 Escherichia coli infection in newborn calves by non-specifically activating the innate immunity (Radoslaw *et al.*, 2017).
- Baypamun™, an inactivated preparation of Orf virus (Parapoxvirus ovis), was demonstrated to greatly decrease clinical illness and virus shedding immediately before to, or in the early stages of infectious bovine rhinotracheitis infection (Castrucci *et al.*, 2000).
- Supplementing preweaned calves with a *S. cerevisiae* fermentation product improves the outcome of Salmonella or Cryptosporidium challenges; and lowers the size and quantity of liver abscesses in finishing beef bulls, with effectiveness equivalent to standard in-feed antibiotic regimens (Shen *et al.*, 2019). To establish innate memory, mechanisms contributing to greater disease resistance, more in-depth examinations of innate cell activity, as well as the particular epigenetic and metabolic modifications that follow these changes, will be necessary.

Sheep and Goats

β-glucans are the most investigated immunomodulatory compounds in sheep,

- Oral supplementation of β-(1-3) (1-6)-glucans to ewes enhances reproductive success as well as offspring development rate and body composition, probably due to β-glucan supplementation's beneficial effects on milk supply and composition in nursing ewes. Monocytes and neutrophils isolated from lambs fed β-glucans had higher phagocytic and respiratory burst activities, as well as lysozyme activity. Lactating ewes given β-glucans had lower somatic cell counts in their milk, and an intramammary infusion of β-glucans caused preferential recruitment of CD14+ monocytes/macrophages to the udder, possibly preparing the animal to be more resistant to mastitis (Waller *et al.*, 1999).
- *Debaryomyces hansenii*, a marine yeast, and its cell wall have been demonstrated to have the ability to educate the innate immunity in newborn goats. Newborn kid goats treated with purified β-glucans from *D. hansenii* and then challenged with LPS show elevated plasma concentrations of TNF-α, IL-6 and IL-1, as well as enhanced respiratory burst activity and nitric oxide generation in isolated leukocytes (Angulo *et al.*, 2020).

As a result, it appears that sheep and goats' innate immune systems may be taught, and the method has tremendous potential for increasing disease resistance in small ruminants.

Swine

β-glucans are widely utilised in commercial pig in-feed products across the world, and they have been shown to provide health advantages.

- If pig diets are supplemented with β-glucan, peripheral blood mononuclear cells generate less pro-inflammatory cytokine in response to LPS stimulation (Li *et al.,* 2006).
- Pigs given inactivated *Mycobacterium paratuberculosis* vaccination showed improved pathological and inflammatory responses after being challenged with *Actinobacillus pleuropneumoniae*, indicating a heightened secondary response due to innate training (Jensen *et al.*, 2019).

BCG is expected to generate innate memory in pigs due to the conserved elements of innate immunity across species, however this has yet to be convincingly established.

Poultry

In contrast to the adaptive immune response, innate memory's rapid induction and breadth provide a key method for reducing illness and foodborne pathogens in chicken.

- The most well-studied of the known innate memory immunostimulants in poultry are β-glucans. Yeast as a β-glucan supplementation inhibits Salmonella colonisation of the cecum (Shao *et al.* 2013) and visceral organs in chicks (Lowry *et al.*, 2005).
- Intermittent feeding of a yeast product containing β-glucans reduced the effects of transportation stress in turkey poults and tended to reduce ceca colonisation with foodborne pathogens Salmonella and Campylobacter (Huff *et al.*, 2010).

Immunostimulants have recently risen to the forefront of in ovo usage as a means of non-specifically boosting the immune system of chickens prior to hatch. The injection of Resiquimod, a TLR7/8 agonist, in the duodenum, lungs, trachea and large intestine of chicks at day 18 embryo enhanced MCR1L-B positive macrophages in the lungs, trachea, duodenum and large intestine at hatch. In ovo delivery of CpG DNA lowered mortality and clinical scores in day old chicks with experimental E. coli infection of yolk sacs. Indeed, Victrio^{R}, a commercial medication advertised to lower mortality in E. coli embryonated eggs and chicks, has been demonstrated to activate TLR21 on chicken macrophages and boost nitric oxide production Ilg T (Abdul-Cader *et al.*, 2019).

Implementing Innate Modulation in Food Animal Agriculture

The major goal of innate immunomodulation in food animals is to improve the animal's immunological condition and resistance to illness in order to improve animal welfare and production efficiency. Antibiotics and the amount of feed necessary to raise an animal to market weight might be reduced if the animal's capacity to withstand sickness is improved. In most production systems, there are clearly defined times when animals are at high danger of infection. The sensitivity of the newborn or very young animal to illness is universal to all production methods (Kollmann *et al.*, 2017). There is a window of increased sensitivity to illness while maternal immunity wanes and an infant's adaptive immune system is untrained.

Although the adaptive immune system in newborns is not completely developed, the innate immune system is active and plays an important role in immunological responses at this age, making it a viable target for increased protection.

A species' duration from birth to market can range from weeks to years; for example, broilers are sold at roughly 6 weeks of age, pigs at 6 months, and beef cattle at 2 years. Thus, the length of protection, as well as how rapidly innate training protection manifests in the animal, is a crucial issue for harnessing innate training.

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Inducing intrinsic training in the newborn period would be predicted to boost disease resistance for at least 6 months, when the "window of vulnerability" to infection is at its most. The first 50 days on feed when calves are weaned and placed in the feedlot, is the next high-risk period for beef. During the transition phase three weeks before to calving to three weeks after calving, dairy cattle are known to be immunosuppressed, resulting in an increase in the incidence of infectious and metabolic disorders. Using the impacts of inherent training or tolerance during the periparturient phase to improve cow health and performance might be advantageous.

Another advantage of innate modulation in both broiler and layer hens is evidence of transgenerational epigenetic modifications to innate immune phenotypes and genes (Berghof *et al.*, 2013).

Potential Pitfalls of Innate Training

Although the idea of using inherent training to improve disease resistance is tempting, there are several possible dangers to be aware of.

- While higher immune responses may be useful for pathogen clearance in food-producing animals, an elevated inflammatory response may cause tissue damage.
- It's possible that boosting this natural inflammatory response even more isn't the best approach. However, if intrinsic training can limit organism shedding, it may still be beneficial to the herd's health by lowering the likelihood of disease transmission.

In order to identify the risk vs. benefit of engaging innate memory, the impact of innate training on tissue pathology, pathogen load, and overall illness outcome will need to be thoroughly examined in the context of specific disease scenarios. Activation of the immune system costs an organism a lot of metabolic energy, which is energy that might be used to make muscle or milk. Activation of cells for cytokine and acute phase protein production and release, as well as cellular proliferation, higher quantities of glucose, amino acids and energy is needed (Rauw, 2012).

Although there is currently little data to support this, it is almost likely that inducing and maintaining trained innate immune cells comes at a catabolic cost to the animal. To fully understand these possibilities, more research will be needed into the efficacy of innate training in avoiding sickness in food animals, as well as the effects of training on animal performance and growth. To mitigate the effects of antimicrobial resistance, measures that improve disease resistance without the use of antibiotics should be considered.

Summary and Conclusions

Only a few research have looked specifically into learned immunity in food animal species. However, as mentioned below, a large body of research supports the use of a number of immunostimulants in cattle, swine, poultry, and small ruminants to improve non-specific, heterologous protection against bacterial and viral illness. Innate memory offers an intriguing prospect to prevent or restrict sickness in agricultural animals, as well as minimise antibiotic use and AMR. Inherited memory has the potential to minimise disease burden and antibiotic use in animal agriculture, and we believe this is one of the most interesting areas of research for a new generation of scientists. To understand how changes in the innate responsiveness of diverse cell types may contribute to disease resistance at the organismal level, more study is needed.

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