



EVIW 2024 Abstract Book – All Presentations including Posters

The multiple burdens of zoonoses in low- and -middle income countries (LMICs): why zoonoses are worse for the poor

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Abstract

Introduction: Poor people in LMICs have greater exposure to zoonoses through livestock keeping; living in agricultural communities; interactions with peri-domestic and wild animals; less access to clean water; and, greater vulnerability to climate shocks. Although their consumption of animal source products is low, the quality of these products is poor. In addition to human health burdens, zoonoses reduce livestock productivity and are important barriers to trade in livestock products, as well as causing more difficult to quantify harms such as spillover to wildlife populations.

Methods & Results: Assessing the impacts of zoonoses helps prioritize management. I present a typology for zoonoses according to epidemiology and argue that although epidemic zoonoses are more dreaded, endemic zoonoses have more negative impact on the poor. Among the most important zoonoses in LMICs are leptospirosis, cysticercosis, brucellosis, tuberculosis, and rabies and zoonoses causing foodborne disease. The COVID-19 pandemic also showed how lack of resilience leads to greater vulnerability of poor people to emerging zoonoses of high economic impact.

Conclusion: I argue investment and innovation is urgently needed to tackle zoonoses in developing countries where they currently impose massive burdens on human, animal and ecosystem health and summarize major advances in approaches to understanding and managing the zoonoses of poverty in the last decade.

Keywords

Zoonoses, low and middle income countries

Meeting the challenge of controlling viral immunopathology

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Abstract

Abstract: The mission of the talk is to identify immune damaging participants involved in antiviral immunoinflammatory lesions. We argue these could be targeted and their activity changed selectively by maneuvers that at the same time may not diminish the impact of components that help resolve lesions. Ideally, we need to identify therapeutic approaches that can reverse ongoing lesions that lack unwanted side effects and are affordable to use. By understanding the delicate balance between immune responses that cause tissue damage and those that aid in resolution, novel strategies can be developed to target detrimental immune components while preserving the beneficial ones. Some strategies involve rebalancing the participation of immune components through various approaches, such as removing or blocking proinflammatory T cell products, expanding regulatory cells, restoring lost protective cell function, using monoclonal antibodies to counteract inhibitory molecules, and exploiting metabolic differences between inflammatory and immuno-protective responses. These strategies can help reverse ongoing viral infections. We explain various approaches, from model studies and some clinical evidence that achieve innate and adaptive immune rebalancing offering insights into potential application to controlling chronic viral induced lesions.

Keywords

immunopathology, virus, immunometabolism, immune mechanisms, immunotherapy

Discovery of neutrophil subsets in cattle

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Abstract

For a long time, neutrophils were only considered as microbe killers. However, their role is far more complex as cross talk with T cells or dendritic cells have been described for human or mouse neutrophils. In cattle, we identified a new subset of regulatory neutrophils that circulate in blood under steady-state conditions. These regulatory neutrophils that display MHC-II are morphologically indistinguishable from classical MHC-II^{neg} neutrophils. However, regulatory and classical neutrophils display distinct transcriptomic profiles and only MHC-II^{pos} are able to suppress T cell proliferation under contact-dependent mechanisms. We investigated the roles of this newly discovered neutrophil subset during two major cattle diseases: mastitis and bovine tuberculosis.

During mastitis, the increase in milk somatic cell count is mainly due to neutrophils influx, which have a crucial role in the elimination of pathogens. We will discuss the implication of classical and regulatory neutrophils in the mammary gland immunity during clinical and subclinical mastitis.

Regarding tuberculosis, neutrophils are implicated in the process of killing *Mycobacteria* early during infection, but also contribute to the chronic inflammation and lung tissue destruction. These dual roles are documented in Human and mouse, and associated to different subsets. With the identification of regulatory neutrophils in cattle, we must now decipher their contribution to the bovine tuberculosis physiopathology.

Our results emphasize distinct contribution of neutrophils subsets for pathogen control and place regulatory neutrophils as true partners of the adaptive immune response. They open the way for discovery of new biomarkers and therapeutic interventions to better control cattle diseases.

Keywords

Neutrophils, cattle, infection

Intriguing immune escape mechanisms developed by three animal viruses: equine herpesvirus 1, porcine reproductive and respiratory syndrome virus and feline enteric coronavirus

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Abstract

The immune system is complex. The reason for this is the increasing variety of tricks that pathogens have developed over time to escape from immunity. Every time a new immunological branch was developed, pathogens tried to find a way to overcome it. This never-ending fight is called co-evolution. Invertebrates have an exoskeleton and are very well protected by strong barriers (cuticle). They only possess an innate immunity and did not need to develop an adaptive immunity. In contrast, vertebrates have an endoskeleton and weak barriers at the interface with the environment (mucus). They had a hard fight to survive during evolution, with pathogens continuously attacking their mucosae. They had to develop an adaptive immunity in addition to the innate immunity.

The mechanisms that three vertebrate viruses developed to escape from immunity will be presented:

- Despite the presence of neutralising antibodies, equine herpesvirus type 1 hijacks monocytes and T-lymphocytes during infection of the upper respiratory mucosa, allowing the virus to reach and affect internal target organs (CNS, uterus) via the blood. The infected leukocytes behave like stealth bombers.
- Porcine reproductive and respiratory syndrome virus is persisting for a long time in its host. It escapes from immunity by (i) using sugars to bind and internalise its target cell (macrophage), (ii) reducing the induction of neutralising antibodies, (iii) spreading between cells through unrecognisable apoptotic bodies, (iv) changing envelope proteins by mutation.
- Feline enteric coronavirus is escaping from local neutralising antibodies in the intestines through an ingenious escape strategy and allows FIP development.

Keywords

virus, immune escape, vertebrate

Circadian rhythms and their importance in regulating the immune response

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Abstract

The immune system is highly time-of-day dependent, which governs the strength of adaptive immune responses, even weeks after antigen exposure. In this presentation, I will provide a global overview of the circadian immune system, focusing on recent advances in the rapidly expanding field of circadian immunology. I will specifically discuss data detailing how vaccine responses are time-of-day gated, against microbial and tumor antigens, and how this might be harnessed to optimize immunotherapies.

Keywords

circadian; vaccination; cancer

Deciphering vaccine immunogenicity pigs promotes vaccine development for both pigs and humans

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Abstract

“The usefulness of swine as an animal model for vaccine development is strongly limited by its immunological toolbox.” This critique challenged many swine researchers 10-15 years ago. Over this time however, the swine immunology toolbox has greatly improved. Nowadays, a plethora of state-of-the-art immunological methods are available: e.g., single-cell RNAseq, tetramer technology, and multi-color flow cytometry enable a detailed analysis of the cellular immune response; ELISAs and other methods facilitate the quantification of antibodies, their avidity and neutralization ability; and qPCR and cell culture-based methods permit a detailed pathogen quantification to evaluate vaccine efficacy. This porcine toolbox is more than adequate for vaccine development. Combined with their high similarities to humans, swine are a valuable animal model for vaccine development. To optimally use its model, swine should be incorporated into a pre-clinical biomedical research pipeline: With an excellent toolbox and affordability, mice can drive antigen and adjuvant discovery; the swine model can then be used to validate murine results and increase translatability. This pipeline will facilitate the progression of vaccine candidates into clinical trials and hopefully provide essential vaccines against human pathogens. Lessons learned from this pipeline can then be applied to vaccine development for porcine diseases. With this One Health approach, the use of the swine model for vaccine development can profoundly benefit the health of both pigs and humans. This presentation will provide an overview of how research in humans, mice and swine can be combined to drive vaccine development.

Keywords

animal model, vaccine development, One Health

Harnessing mucosal immunity: lesson from the pig model

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Abstract

Influenza virus infection remains a global health threat to humans and livestock. There is an urgent need to develop novel control strategies and vaccines that prevent transmission and provide broader protection. We investigated how best to prevent transmission and induce local immunity, using pigs, which are an important natural host for influenza, are a source of pandemic viruses, and are an excellent model for human disease.

We used scintigraphy to evaluate the distribution of droplets in the respiratory tract following different methods of antigen delivery and compared the protective efficacy of influenza vaccine candidates following intra-nasal, aerosol and intra-muscular immunization. We analyzed antigen specific T cell responses in lymphoid and non-lymphoid tissues of inbred Babraham pigs following immunization or infection and show that the specificity of the responses in blood does not reflect that in the respiratory tract. We demonstrated that the rates of cell proliferation and decline of phenotypically defined CD8 subsets, including tissue resident memory T cells, are comparable between lymphoid and non-lymphoid tissues. We defined the contribution of antibody and T cells in protection following immunization with viral vectored vaccines administered systemically or parenterally. These results will inform how best to deliver vaccines in order to harness optimal mucosal immunity and provide valuable insights into the antigen composition of broadly protective vaccines in a highly relevant large animal model.

Keywords

influenza, pig, mucosal immunity, tissue resident memory cells

Solving the puzzle of mononuclear phagocytes – species by species

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Abstract

Across species, dendritic and monocytic cells have evolved to serve as innate sensors, inflammatory effectors, and instructors of adaptive immunity. The high plasticity of monocytic cells and their transcriptomic overlap with bona fide dendritic cells and macrophages further supports the fundamental importance of this system but makes it notoriously difficult to study.

Despite limitations, single-cell transcriptomics is the most promising approach to capture diversity and trajectories, however the challenge of differentiating subsets remains. So rather than forcing subset definitions, we may investigate gene expression/ clustering patterns across species. With these comparative analyses we aim to elaborate fundamental features of the mononuclear phagocyte system (MPS), but also reveal species-specific adaptations.

By performing in-depth scRNA-seq analysis on the MPS in bovine blood and lymph nodes, we generated a set of hypotheses and initiated a "puzzle" across species and tissues. In addition to published datasets, we are currently integrating and comparing our scRNA-seq data on dendritic cells enriched from blood of horses and our most recently generated datasets on dendritic and monocytic cells from blood of pigs.

Piece by piece and species by species, this puzzle should improve our understanding of the MPS and eventually offer new possibilities for vaccination and immunotherapy.

Keywords

mononuclear phagocytes, scRNA-seq, bioinformatics

Understanding Mucosal Immunology to Enhance Vaccine Development

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Abstract

The bovine mucosal immune system responds to the transition from sterile fetus to colonization by commensal microbiota. This challenge is met with rapid development of immune competence, encompassing IgA plasma cell differentiation and IgA secretion within the first week of life. This development varies, however, among mucosal sites. Of interest for the control of disease, is rapid onset of IgA responses in the upper respiratory tract (URT) to commensal bacteria that are also opportunistic respiratory pathogens. This provides a unique opportunity for intranasal (IN) vaccination in neonatal calves. This vaccination route circumvents interference by maternal antibody and induces immune responses and immune memory that protect the neonate as passive immunity wanes. Co-vaccination with multiple IN vaccines is now feasible. The enteric mucosal immune system provides a unique opportunity for oral vaccines to induce T regulatory (Treg) cells. Oral vaccination of neonatal calves with a modified-live bacterial vaccine induces Tregs that reduce pulmonary inflammation following opportunistic bacterial infections. Development of conjunctival-associated lymphoid tissue (CALT) is delayed in neonatal calves. Ocular vaccination can accelerate CALT development and enhance IgA secretion in tears. This may provide a strategy to reduce opportunistic bacterial infections causing bovine keratoconjunctivitis. A major challenge remains, however, in developing vaccines targeting persistent enteric pathogens. This requires further research to better understand effects on B lymphocyte development when pathogens infect the primary lymphoid tissue located in the continuous Peyer's patch of the small intestine. A better understanding of mucosal immune system development and regulation will reveal new vaccine opportunities.

Keywords

Bovine, Mucosal Immunity, Vaccines

Canine Immunology: Insights into Polarization of antigen presenting cells and T cell responsivity

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Abstract

Comparative immunology elucidates both unique and shared aspects of immune functions across species. Recently we have developed in vitro model systems to study polarization of canine macrophages and subset characterization of other antigen presenting cells (APCs) as models to study immune responses against infectious diseases. APCs play a pivotal role in the immune responses, acting as a bridge between innate and adaptive immunity. Macrophages, derived from monocytes, are distributed throughout the body, residing in almost all tissues, where they ingest and process antigens.

Canine macrophages can be polarized into various subsets, including the classically activated (M1) and alternatively activated (M2) macrophages, each characterized by distinct cytokine profiles, surface markers, and functions. Using in vitro culture of monocyte derived dendritic cells and macrophages we have studied innate immune activation in different canine-relevant infectious agents.

Our findings reveal distinct morphological and functional characteristics between M1 and M2 polarized macrophages similar to other mammalian species. However, highlights the complexity of studying canine immunology. Transcriptomic and proteomic data further supported these functional disparities, illustrating the complex regulatory networks involved in macrophage and dendritic cells responses to pathogens.

The plasticity of macrophages and their dual roles in promoting and resolving inflammation make them central to both the pathogenesis and resolution of infectious diseases.

These results not only advance our understanding of canine immunology but also reinforce the utility of canines as a model for human infectious diseases, offering potential avenues for therapeutic and vaccine development across species.

Keywords

canine immunology, antigen presenting cells, adaptive immune response, innate immune activation

Gene Editing Opportunities for Livestock Disease

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Abstract

Gene editing (GnEd) involves using a site-directed nuclease to introduce a double-stranded break (DSB) at a predetermined location in the genome to affect a desired characteristic. Many applications of GnEd in animals for agricultural applications are targeting health and welfare traits that are directly relevant to veterinary professionals. These include the generation of disease-resistant animals (e.g. PRRS virus resistant pigs), and applications designed to avoid painful procedures such as dehorning and castration. For editing to be incorporated into livestock breeding schemes, it will need to seamlessly integrate with genetic improvement program design. For this to occur, the efficiency of editing the next generation, through the production of homozygous, non-mosaic developing embryos will have to become routine. Additionally, edits will likely have to be introduced into multiple elite animals to avoid genetic bottlenecks. It will also require editing of multiple individuals in different breeds and lines to maintain genetic diversity, and enable structured cross-breeding. This requirement is at odds with the process-based trigger and event-based regulatory approval approach that has been proposed for the products of GnEd animals in some countries. Other countries, including Argentina, Australia, Brazil, Colombia, and Japan, have adopted a regulatory policy that considers mutations introduced following GnEd DSB repair as akin to natural genetic variation, and therefore treat these GnEd animals analogously to those produced using conventional breeding. Introducing disease resistance and welfare traits offers an opportunity to reduce animal death, suffering, and the use of chemicals to control disease; goals that align with public values.

Keywords

Gene editing, Disease Resistance, Regulations

Immunocellular Dynamics and Metabolic Adaptations in Equine Recurrent Uveitis

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Abstract

Introduction: Equine recurrent uveitis (ERU) poses a significant challenge in veterinary medicine due to its recurring nature and vision-threatening potential. Understanding the intricate interplay between immune cells and ocular cells is crucial for unraveling ERU pathogenesis. This study aims to elucidate these dynamics and metabolic adaptations underlying ERU, leveraging advanced technologies to provide insights into potential therapeutic targets.

Methods: Immune models of ERU were established, and samples from equine subjects were collected for analysis. Immunocellular interactions were investigated through flow cytometry, focusing on T cells and neutrophils. Metabolic adaptations within these immune cells were assessed using Seahorse analysis, examining glycolysis and oxidative phosphorylation pathways. Concurrently, the crosstalk between immune cells and cells within the eye, particularly Müller glia, was explored to elucidate their contribution to disease progression.

Results: Our findings reveal dysregulated activation of T cells and neutrophils in ERU, accompanied by pronounced metabolic shifts towards enhanced glycolysis and altered oxidative phosphorylation. Furthermore, we observe significant crosstalk between immune cells and ocular cells, implicating Müller glia in the pathogenesis of ERU.

Discussion/Conclusion: The immunocellular dynamics and metabolic adaptations uncovered in this study shed light on the complex mechanisms driving ERU pathogenesis. By elucidating these intricate pathways, potential therapeutic targets for mitigating vision-threatening complications in affected equines are identified. This research underscores the importance of integrating advanced technologies to enhance our understanding of ocular inflammatory diseases and develop targeted therapeutic interventions.

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Keywords

Equine recurrent uveitis, immunocellular dynamics, metabolic adaptations

Using deep learning to classify bovine retinal images into tuberculosis and non-tuberculosis disease states

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Abstract

Purpose

This study aimed to validate an artificial intelligence-based deep learning system to classify tuberculosis (TB) test positive (TBTP) cattle using retinal imaging. The deep learning system was compared to a set of images graded for disease by three veterinary ophthalmologists.

Methods

The study sample consisted of TBTP cattle in England and Wales, and cattle based in Scotland (an officially TB-free area (OTF)). Retinal images were collected *post-mortem* using an Optos California ultra-wide field retinal camera. In total, 1570 images were captured from 870 animals, 575 of which were TBTP and 295 were OTF. The data was divided manually into 'train', 'validation', and 'test' sets with a ratio of 70/15/15, ensuring that no individuals appeared in more than one set. In MATLAB, three deep learning-based neural networks were trained to classify the images as either healthy or diseased; ResNet50, MobileNet-v2, and Xception.

Results

Xception was the best performing model, achieving an accuracy of 94.0% on the 'test' set (235 images, 127 cows). The sensitivity was 98.8% and the specificity was 85.0%. By comparison, the manual gradings were 45-51% accurate, with a 16-52% sensitivity and 37-83% specificity.

Conclusion

The study shows that a deep learning system can detect TB in the eyes of cattle with a much higher accuracy than conventional assessment.

Keywords

Deep-learning, tuberculosis, bovine

Teleost fish IgM⁺ plasma-like cells: beyond antibody secretion

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Abstract

Upon antigen encounter, B cells start a differentiation process towards antibody secreting cells (ASCs), initially plasmablasts, and eventually long-lived plasma cells (PCs). All these ASCs specialize in secreting important amounts of antibodies and usually lose other functionalities of naïve B cells. This differentiation process is scarcely characterized in teleost fish, where B cells have been shown to share many functional and phenotypic characteristics of mammalian B1 innate subsets. In this context, we were prompted to investigate further the functionalities of ASCs in teleost, using rainbow trout (*Oncorhynchus mykiss*) as a model. Our results demonstrate that IgM⁺ plasma-like cells in the rainbow trout head kidney (HK) exhibit a strong IgM secreting capacity along with phagocytic and antigen-presenting capacities, even higher than those of naïve B cells. These IgM⁺ plasma-like cells were capable of surviving *in vitro* for 2 weeks secreting IgM. Interestingly, they retained a functional B cell receptor (BCR) that responded to TNP-LPS, a thymus-independent (TI) model antigen, which also rendered these cells more reactive to BCR cross-linking. These findings shed light on the differentiation process of teleost B cells, demonstrating that teleost plasma-like cells conserve other phenotypical attributes beyond immunoglobulin secretion, being capable of directly responding to antigens. These findings point to an exclusive differentiation process of teleost B cells, which might provide mechanistic insights on how mammalian innate subsets such as B1 cells or IgM-expressing PCs differentiate.

Keywords

fish; B cells; plasma cells

Targeted delivery of oral vaccine antigens to aminopeptidase N protects pigs against pathogenic *E. coli* challenge infection

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Abstract

Introduction:

Many pathogens enter the host via the gut. Despite being best suited for eliciting intestinal immunity, oral vaccination remains challenging due to the harsh gastrointestinal environment, a poor uptake of vaccine antigens by the intestinal epithelium and the tolerogenic environment pervading the gut. To circumvent these hurdles, we have developed a novel oral vaccination strategy that specifically targets vaccine antigens towards aminopeptidase N (APN), a membrane receptor present on small intestinal enterocytes that is known for its role in mediating epithelial transcytosis.

Methods:

To target vaccine antigens towards APN, the FedF tip-adhesin from F18 fimbriated *E. coli* was genetically linked to the Fc-domain of an APN-specific porcine IgA antibody. This antibody-antigen fusion construct was orally administered to piglets and immune responses were followed up. After vaccination, the piglets were orally challenged with an F18+ *E. coli* strain to assess protective efficacy.

Results:

Oral vaccination with the APN-targeted antibody-antigen fusion construct resulted in robust mucosal and systemic immune responses and provided protection against a subsequent challenge infection with the F18+ *E. coli* strain.

Conclusion:

Altogether, this study demonstrates the effectiveness of oral vaccine delivery by targeting antigens towards Aminopeptidase N, overcoming several challenges associated with traditional oral vaccination approaches. These findings suggest that by exploiting APN-mediated transcytosis, antigens can effectively reach the gut immune system, leading to enhanced protection against gut pathogens. This study provides a solid foundation for the development of new oral subunit vaccines to protect against gut pathogens using APN-mediated delivery of vaccine antigens.

Keywords

Oral vaccination, mucosal immunity, subunit vaccine

IN VITRO PHAGOCYTOSIS COMPETITION: A TURTLE, A GOOSE AND A DOG – WHO HAS THE BEST SCORE?

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Abstract

Phagocytosis is an effective innate immunity mechanism, that activates at the host's initial contact with pathogen or foreign particle. In mammals' blood, it is dominantly performed by neutrophils and monocytes. Avian and reptilian phagocytic potential is assisted by few other recognized phagocytes: heterophils equivalent to mammalian neutrophils, eosinophils, B lymphocytes, red blood cells and thrombocytes. The aim of this study was to compare *in vitro* phagocytosis by leukocytes, between three animal classes: reptiles, birds and mammals. Each group included six healthy animals of following species: turtle, goose and dog. Heparin blood samples (30 µL) were incubated with opsonized zymosan particles (6 µL, 1 mg/mL) for 15 minutes at 37°C and 15 minutes at room temperature, respectively. Differential leukocyte formula was assessed microscopically, using Romanowsky-stained blood smears. *In vitro* functional ability was compared based on the equivalent types of leukocytes that performed phagocytosis, as well as total phagocyte capacity (%) *per* 100 detected leukocytes on a blood film. The results presented neutrophils as the most active in canine blood ($P=0.001$), when compared with heterophils in turtles and geese. The turtles had both highest monocyte ($P=0.002$) and total phagocyte capacity ($P=0.002$), in comparison with the other two species. Total leukocytes were the highest in geese ($P=0.003$), but their active phagocytes had the least activity towards zymosan particles. Not only heterophils and monocytes, but also eosinophils, basophils and lymphocytes displayed phagocytic ability in turtles, indicating that reptile leukocytes have the most preserved phagocytosis potential across leukocyte types and among analyzed animal classes.

Keywords

immunity, leukocytes, zymosan

Improving the early immune parameters of lung transplantation in the pig model by conditioning donors with corticosteroids.

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Abstract

Lung transplantation (LT) is an increasingly applied cure of terminal respiratory diseases, despite frequent disappointing outcomes. The innate allogeneic immune response upon LT is implicated in the induction of the detrimental primary graft dysfunction and of rejection events. Therefore, targeting the innate allogeneic response with an optimal treatment is highly desirable. The standard of care includes a methylprednisolone bolus administered to the recipient. However, there is no consensus about the potential benefit of adding this same treatment to the donor. In the pig model, we used a cross-circulatory platform that consists in a donor lung placed extracorporeally and connected to the circulation of a perfusing pig whose leukocytes are fluorescent. We compared the early immune parameters in the perfused lungs of 3 groups of pig pairs: i) untreated donors and recipients, ii) treated recipients only, iii) treated donors and recipients. Interestingly we found that treating the donor reduced the initial CD3 T-cell representation in the graft as well as the recruitment of recipient CD3 T-cells, and enhanced the anti-inflammatory profile of the alveolar macrophages. The proportion of donor and recruited B and NK cells was not altered. Treating the recipient, independently of the donor treatment, reduced the inflammatory profile of the recruited monocytic CD14+ and CD16+ subsets. The donor treatment's effect was related to a decrease in T-cell chemokine gene expression in the graft (CXCL9, 10 and 11). These results indicate that treating both the donor and recipient with corticosteroids improve the early immunological status of lung grafts.

Keywords

lung, transplantation, pig model

Molecular characterization of immune related genes in native Poonchi chicken from Jammu, India

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Abstract

Introduction: Poonchi is one of the remotest districts of the Jammu and Kashmir (UT) and situated on international borders. Backyard poultry farming with local native indigenous chicken is common practice in these hilly areas. This native poultry population is quite hardy and considered to be more disease resistant. Present study was conducted for characterization of *ChB6* (Chicken B-cell marker), *IAP-1* (inhibitor of apoptosis protein-1) and *IL-15R α* (Interleukin-15) genes in indigenous Poonchi chicken population.

Material and Methods: RNA was extracted from fresh blood samples of Poonchi chicken and Kadaknath chicken. Direct sequencing was carried out by Sanger sequencing and results were analyzed using BioEdit and MEGA X software's.

Results: The results obtained from sequence analysis showed that there was no variation in the sequences of Poonchi, Kadaknath and Leghorn chicken populations for *ChB6* and *IAP-1* genes but there was variation with Fayoumi and other reported *Gallus gallus* sequences and other species. For *IL-15R α* gene there was variation within Poonchi population as well as between different chicken populations like Kadaknath, Leghorn, Fayoumi. For *IL-15R α* in the present study T \rightarrow C SNP change was detected. The highest genetic distance for *ChB6* gene was observed between Poonchi chicken and *Miniopterus fuliginosus*. The highest genetic distance for *IAP-1* gene of Poonchi chicken was observed with *Tympanuchus phasianellus*. For *IL-15R α* gene within the Poonchi chicken population the genetic distance was 0.0078.

Conclusion: The studies indicate that there is sustainable variation between the different breeds and species. Therefore, further association studies in large population would be helpful to identify disease resistant / susceptible genotypes chicken population.

Keywords

ChB6, IAP-1, IL-15R α , Poonchi chicken

Evaluation of Live Attenuated Porcine Reproductive and Respiratory Syndrome Virus Vaccines Genetically Engineered to Express Peptide-based Immune Checkpoint Inhibitors

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Abstract

Background: Porcine reproductive and respiratory syndrome viruses (PRRSV) cause significant economic losses in the global pig industry. Live attenuated PRRSV vaccines (MLV) are widely used, but are weakly immunogenic and provide limited protection, possibly due to the modulation of immunoregulatory pathways. This study tested the hypothesis that engineering PRRSV MLV to express immune checkpoint inhibitors (ICI) could enhance the induction of protective responses in pigs.

Methods: Three PRRSV-1 MLV were engineered by reverse genetics to express peptide-based ICIs: (i) Microtides LD01 and (ii) LD10, which are both PD-1 and CTLA-4 dual antagonists, and (iii) 4ZQK13_Pig20, a synthetic porcine PD-1 binder protein. MLV-ICI were rescued and characterised *in vitro*. The safety, immunogenicity, and efficacy of the MLV-ICI were then compared against the parental recombinant MLV in piglets.

Results: All three PRRSV-1 MLV-ICI were successfully rescued and propagated in MARC-145 cells. Immunisation of piglets with PRRSV MLV-ICI showed no adverse effects. Compared to the parental MLV and other MLVs expressing ICI, immunisation with the MLV expressing Microtide LD10 showed a trend towards greater IFN- γ and virus-neutralising antibody responses. Following challenge with a PRRSV field strain, pigs vaccinated with MLV expressing Microtide LD10 showed significantly reduced viral loads in the lungs compared to the other MLVs.

Conclusions: Novel PRRSV-1 vaccine candidates have been constructed that express peptide-based ICI. The LD10 candidate exhibited the greatest potential and provides a basis for future research on optimizing peptide ICI-based adjuvant approaches for better control of PRRSV and other viral infections of swine.

Keywords

PRRSV, Vaccinology, Immune checkpoint inhibitors.

***Bos taurus* and *Bos indicus* cattle exhibit compelling different immune responses towards vector-borne viruses**

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Abstract

Cattle are the mammalian species with most global biomass associated with a huge impact on Earth. In line with the 3R principles, we developed an *ex vivo* platform using fresh primary bovine blood cells to characterize and dissect host-pathogen interactions. This procedure employs a novel multiparameter flow cytometry assay (measuring maturation / activation of most immune cell subsets) and a multiplex immunoassay (monitoring chemokine / cytokine secretions). We hypothesize that *Bos taurus* and *Bos indicus* genetic background are likely to show different immune responses and susceptibilities towards vector-borne diseases (VBDs) because of their different evolutionary trajectory and origin of domestication. Here we tested our hypothesis using two vector-borne viruses, namely Bluetongue virus (BTV) and Schmallenberg virus (SBV), both expected to trigger increasing numbers of outbreaks in Europe in the future due to climate change. When only considering *Bos taurus*, we found an *ex vivo* response towards SBV very moderate compared to BTV; this clearly indicates a fine-tuning of the bovine immune response depending on pathogen. The most striking finding was the genetic-borne differential response towards BTV. *Bos taurus* exhibited an enhanced activation of monocytes, dendritic cells, CD8 and $\gamma\delta$ T cells, whereas *Bos indicus* relied mostly on CD4 T cells. Overall, we provide novel insights in the immune responses of cattle with markedly different genetic background to VBDs. Our platform can be applied to test immune responses of different cattle breeds and pinpoint immune responses that might confer protection and assist breeding programmes and vaccine development.

Keywords

Vector-borne diseases; Cattle breed; RNA vector-borne viruses; Disease susceptibility; Ex vivo immune response, Bluetongue virus, Schmallenberg virus

Comparative analysis of swine leukocyte antigen (SLA) gene diversity in Göttingen Minipigs

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Abstract

Worldwide, pigs are economically important farm animals, also representing a preferred preclinical large animal model for biomedical studies. The need for swine leukocyte antigen (SLA) typing is increasing with the expanded use of pigs in translational research, infection studies, and for veterinary vaccine design. Göttingen Minipigs attract increasing attention as valuable model for pharmacological studies and transplantation research. This study represents a first-time comparative metadata analysis of SLA gene diversity in Göttingen Minipigs with commercial pig lines. In 209 Göttingen Minipigs, SLA class I and class II genes were characterized by PCR-based low-resolution (Lr) haplotyping. Criteria and nomenclature for SLA haplotyping were proposed by the ISAG/IUIS-VIC SLA Nomenclature Committee. Haplotypes were assigned based on comparison with already known breed or farm-specific allele group combinations. In total, 14 SLA class I and five SLA class II haplotypes were identified in the studied cohort, to manifest in 26 SLA class I but only seven SLA class II genotypes. The most common SLA class I haplotypes Lr-24.0 and Lr-GMP-3.0 occurred at frequencies of 23.44 and 18.66%, respectively. For SLA class II, the most prevalent haplotypes Lr-0.21 and Lr-0.03 occurred at frequencies of 38.28 and 30.38%. The comparative metadata analysis revealed that Göttingen Minipigs only share six SLA class I and two SLA class II haplotypes with commercial pig lines. More importantly, despite the limited number of SLA class I haplotypes, the high genotype diversity being observed necessitates pre-experimental SLA background assessment of Göttingen Minipigs in regenerative medicine and xenograft research.

Keywords

swine leukocyte antigen (SLA), polymorphism, Göttingen Minipigs, xenograft

PRRSV-induced T Cell Responses at the Maternal-Fetal Interface During Late Gestation

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Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) is an RNA virus that causes reproductive failure, especially during late gestation. The *in utero* immune responses remain poorly understood. Previous findings demonstrated an increase of T cells with effector phenotypes at the maternal-fetal interface following infection. This might have affected tissue integrity since this increase was limited in vaccinated gilts. In a new experiment, we investigated the phenotype and functionality of local T cells following infection. Pregnant gilts (n = 5) were infected with the PRRSV field isolate AUT15-33 or sham-inoculated (n = 5) at day 85 of gestation. Twenty-one days later, gilts were euthanized and mononuclear cells from the maternal endometrium (ME) and fetal placenta (FP) were isolated. T cell phenotypes and their Ki-67 expression as well as PRRSV-specific T cell responses were assessed. In infected gilts, we observed an increase in Ki-67⁺perforin⁺CD27^{dim} effector CD8 β ⁺ T cells and Ki-67⁺CD8 α ⁺CD27⁺ central memory CD4⁺ T cells, at both sides of the maternal-fetal interface, as compared to the controls. Furthermore, following restimulation with PRRSV AUT15-33 peptide pools, we were able to detect PRRSV-specific CD4 and CD8 β T cells, based on intracellular cytokine staining for IFN- γ , TNF- α , and IL-2. These data suggest that local T cells, especially CD8 T cells, even in the separated FP respond to PRRSV-infection, but their activation may cause immunopathogenesis. Further functional and *in situ* investigations are required to corroborate this hypothesis.

Keywords

T cells, PRRSV, maternal-fetal interface

Insight into genetic background of mastitis resistance in cattle: a comprehensive collection of candidate loci

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Abstract

Our research addresses the problem of mastitis in dairy ruminants, which has a detrimental effect on milk production, animal welfare and antimicrobial resistance. Selective breeding for higher milk yield has led to an increase in the prevalence of mastitis, so there is a need to improve natural mastitis resistance in dairy ruminants. However, mastitis resistance is a complex trait, making the identification of mastitis-associated genes in livestock a difficult task. To assist researchers in the selection of candidate genes for further functional validation studies, we have compiled a comprehensive database of candidate genes for mastitis resistance.

Extensive data collection was performed, *i.e.* extraction of mastitis-associated candidate genes from the relevant literature, followed by subsequent prioritisation analysis, pathway enrichment, and protein-protein interaction analysis.

Our research integrated multiple data sources to collect 2452 candidate genes associated with mastitis resistance. The candidate loci are evenly distributed across all bovine chromosomes, with a high concentration of markers observed on chromosomes 5 and 9. The list of the most promising candidate genes contains 22 genes, including well-known genes associated with the immune response (*e.g.* *CXCL8*, *CXCR1/2*, *TLR2/4*), which could be considered a priority for validation studies.

Our database confirms the complexity of the trait and provides an overview of the genetic background of mastitis resistance. In addition, we have established several primary bovine mammary epithelial cell lines from milk that will serve as *in vitro* models for validation studies of the proposed candidate genes.

Keywords

Candidate genes; Mammary gland; Mastitis

The presence of chronic subclinical systemic inflammation during remission of canine atopic dermatitis

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Abstract

Canine atopic dermatitis (CAD) represents a common, progressive, chronic, pruritic, inflammatory disorder. As genetic and environmental factors jointly disrupt epidermal barrier functions, immune dysregulation occurs, followed by cutaneous microbiome alterations. The question of whether skin injury in CAD elicits systematic response remains open. We hypothesized that changes in positive and negative acute-phase reactants (APR) could evidence systemic inflammation in CAD. The study included dogs with CAD in remission (n=54), and healthy control dogs (n=26). Blood samples were collected outside the pollination season, in November 2022. Laboratory analyses included routine hematology and biochemistry parameters, as well as determination of the levels of serum amyloid A (SAA), ceruloplasmin (Cp), paraoxonase-1 (PON-1), iron, and copper. Both of the tested groups had hematology and biochemistry values within the reference intervals. However, dogs with atopic dermatitis had higher hematocrit (P=0.026) and eosinophil count (P=0.002), higher total proteins (P=0.018) and globulins (P=0.002), but lower albumin/globulin ratio (P=0.032) and cholesterol (P<0.001), in comparison with control dogs. The CAD group showed a higher concentration of Cp (P=0.006) compared to the controls. Other parameters were within the intervals and did not differ between groups: level of PON-1 (P=0.747), SAA (P=0.926), iron (P=0.532) and copper (P=0.713). Study results demonstrated that dogs with atopic dermatitis in remission have evidence of systemic inflammation, represented by increased Cp, and without change in the other analyzed positive and negative APRs.

Keywords

ceruloplasmin, eosinophils, paraoxonase-1

Humoral and cellular responses to a parapox virus-vectored antigen, potential for the development of a new vaccine to *Chlamydia abortus*

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Abstract

Introduction / Purpose: A safer, more efficacious vaccine to protect sheep from ovine enzootic abortion (OEA) is needed. *Chlamydia abortus* causes OEA in most countries worldwide. Thelper-1-type cellular responses are strongly associated with protection. Virus vectors, such as the parapox virus Orf, have wide applicability in vaccinology to stimulate humoral and cellular immune responses in a range of species. Here, the objective is to measure cellular and humoral immune responses elicited in sheep immunized with a modified Orf virus vector (OrfV) containing the major outer membrane protein (MOMP) of *C. abortus*, a known protective antigen. The work will assess the potential utility of OrfV-MOMP as a vaccine to OEA.

Methods: Thirty sheep were allocated into three groups of 10 and immunized intramuscularly with: 1) live OrfV-MOMP; 2) inactivated OrfV-MOMP; and 3) served as unvaccinated controls. Serological anti-MOMP IgG and cellular recall responses of peripheral blood mononuclear cells to whole killed chlamydial organisms and a subunit antigen preparation containing MOMP were assessed.

Results: Immunization with live or inactivated OrfV-MOMP induced anti-MOMP IgG but not the unvaccinated controls. Antigen-specific recall responses, characterised by the secretion of interferon-gamma and interleukin (IL)-17A, with very low IL-10 and no IL-4 was detected in both immunized groups, suggesting induction of appropriate potentially protective immune responses.

Discussion/Conclusion: OrfV-MOMP induces humoral and cellular responses that could be protective against *C. abortus* infection in sheep. This prototype vaccine, if efficacious, could lead to a safer chlamydial vaccine removing requirement of pathogen handling and manipulation in its production.

Keywords

vaccine; virus vector; Chlamydia

Moo-ving Beyond Allergies: Insight Into Cow Milk Allergy and a Potential Cure

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Abstract

Background: On June 28, 2017, thirteen-year-old boy Karanbir Cheema died from a slice of cheese that was accidentally thrown on his face at school. Freak accidents like these necessitate the clinical investigation of allergies. Omitting an allergen from one's diet is not enough, lest an allergic person or their family wishes to live with paranoia. Between 1998 and 2018, there have been a three-fold increase in hospital admissions for food-induced anaphylaxis.¹ 40% of the world suffers from an allergy, with cow milk protein allergy being the most common one in children.²

Aims: Having recognized a Eurocentric bias in scientific research, this study aims to narrow this discrepancy by comparing a British cohort with one from Morocco. Bhabab et. al. (2022), for example, emphasised the need for regional data in the Middle East and North Africa (MENA) because of a knowledge gap among physicians in distinguishing CMA from lactose intolerance and other similar conditions.

Hypothesis: The biggest distinction between the two populations will be demonstrated in dietary and environmental variables. These variables will influence the incidence of IgE-mediated CMA amongst infants in Morocco compared to the United Kingdom.

Methods: A systematic literature review that will analyse IgE-mediated cow milk protein allergy (CMPA or CMA) in infants, exploring incidence, diagnosis, and management techniques. Since there is no raw data in Morocco available, a prospective study would have been conducted there.

Preliminary Results: In the UK → over diagnosed. 1% of the pediatric population.⁴ Follows standard BSACI procedure. Management includes omission from diet, oral food challenge, and reintroduction. In Morocco → underdiagnosed. 9.5% suspected in the general population.⁵ Protocol adopted from French system. Management includes milk substitutes.

Conclusion: Morocco has unmet needs because of the under recognition of allergy as a clinical specialty. The UK on the other hand, could benefit from a change in its management and immunotherapy procedures or lack thereof (e.g. early introduction instead of elimination of an allergen). Omalizumab is a viable candidate for long-term treatment.

Keywords

cow milk protein allergy

Identification of immune correlates of protection after intranasal vaccination with the attenuated African swine fever vaccine candidate BA71ΔCD2

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Abstract

African swine fever (ASF) is a deadly swine disease currently causing a pandemic that results in severe economic consequences to the porcine industry. Control of the disease is hampered by the limitation of available vaccines. Live attenuated vaccines (LAVs) are the most advanced prototypes at present. However, advances achieved using LAVs must be complemented with further studies to analyse vaccine-induced immunity. The BA71ΔCD2 LAV, lacking the viral haemagglutinin CD2v gene, induces robust protection against a lethal challenge at 21 days after vaccination. Here, we characterize the onset of protection triggered by BA71ΔCD2 and the immune components associated. Intranasally vaccinated pigs were challenged with the virulent Georgia 2007/1 strain at days 3, 7 and 12 postvaccination. Only the animals vaccinated 12 days before challenge controlled the infection course, showing low virus loads and minor clinical signs. Contrarily, animals vaccinated earlier just showed a minor delay of disease progression. Detection of humoral responses and analysis of whole blood transcriptome signatures reveal that control of infection is associated with the presence of cytotoxic cells and virus-specific IgG before challenge. Moreover, flow cytometry analyses showed that presence of cytotoxic lymphocytes in blood at the moment of challenge is associated with protection, while a broader cytotoxic response including NK cells is marker of severe pathology. The findings contribute to our understanding of protective immunity against ASFV and are relevant in the present

pandemic context, where LAVs are becoming a strategic tool as a vaccine emergency response to be deployed in ASF-affected areas.

Keywords

ASFV, onset of protection, correlates of protection

Exploring porcine models and MHC genetics in xenotransplantation

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Abstract

Pigs have become the preferred preclinical large animal model for biomedical studies, transplantation, xenotransplantation, and regenerative medicine research. Allogeneic transplantation research in pigs has improved the understanding of rejection mechanisms of both host-versus-graft and graft-versus-host disease. Advances in genomics have informed our understanding of the complexity of the immune system and the genes that influence disease and vaccine responses, with the most important being the swine major histocompatibility complex (MHC) genes, the swine leukocyte antigens (SLA). Gene sequencing data have advanced efforts to define the polymorphisms of class I and class II SLA genes, setting the foundation for probing the role of these genes in swine health and disease. Identification and maintenance of important SLA-defined pig lines (e.g., Göttingen Minipigs, NIH/MGH miniature swine model, Westran pigs, Yucatan, or MINI-LEWE and British Babraham pigs) are essential as resources for pig biomedical models. SLA-defined pigs have served as a significant transplantation model and, with gene editing, a potential source of xeno-organs. Improved human SLA class I and II cross-matched genetically engineered pigs could reduce antibody-mediated rejection of pig xenografts in highly HLA-sensitized patients. Histocompatibility testing of pigs needs to be improved in analogy to human allogeneic transplantation. Modifying SLA genes could improve pigs as donors for xenotransplantation. This lecture summarizes the current knowledge of the genomics of the SLA region, with special reference to Göttingen Minipigs and Micropigs, and explores the importance of SLA genes in allogeneic and xenogeneic transplantation together with regulatory frameworks in swine biomedical research.

Keywords

swine leukocyte antigen (SLA), Göttingen Minipigs, animal model, xenotransplantation

Protective immunization against genotype I of African swine fever virus using adenovirus vectored antigens.

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Abstract

African swine fever virus (ASFV) is a lethal haemorrhagic disease of domestic pigs threatening global food security due to the lack of a vaccine to support prevention and control. It is currently widespread across Asia and also present in Europe where it's still spreading to new countries. Cellular immunity plays a pivotal role in protection against virulent ASFV and we have been studying and optimizing immunization strategies using vectored ASFV antigens. Our model of immunization uses pools of adenovirus vectors expressing specific ASFV genes. We showed that these can confer high levels of protection of up to 80% from challenge with virulent ASFV of genotype I. Recently we screened potential new T-cell antigens to improve this level of protection. Immune cells from vaccination experiments with different combinations of vectored antigens were assessed for CD4+CD8 α + T-cell responses after recall stimulation with peptides, leading to the identification of new potentially protective T-cell antigens. These were incorporated in different combinations of antigens in our adenovirus vectored immunizations, resulting in 100% protection with some of the pools and significantly low clinical scores and low or absent viremia after challenge. Significant virus- and antigen-specific T-cell and antibody responses were observed, indicating that both cellular and humoral arms of the immune response were effectively triggered. The levels of protection observed in this study are the best observed so far with our model of immunization and show high promise for development of a future subunit vaccine for ASFV.

Keywords

African swine fever virus, vectored antigens, Immune responses

miR-215 modulates inflammasome activation and autophagy during *Salmonella* infection in porcine intestinal cells

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Abstract

Host response to *Salmonella* Typhimurium (ST) infection in pigs can be post-transcriptionally regulated by miRNAs. Among the several miRNAs that we have reported to be dysregulated during ST infection, we find decreased expression of miR-215.

To evaluate miR-215 function during infection we used *in vitro* mimic transfections in an IPEC-J2 intestinal cell line. LFQ proteomic analysis of infected (ST), transfected (m215) and transfected and infected (m215+ST) cells identified 157 proteins. From them, 35 were downregulated when miR-215 was overexpressed, thus being potential targets of this miRNA. We selected 28 proteins downregulated by miR-215 (targets) and upregulated in ST infection, which were validated by qPCR and/or *western blot*. We found that miR-215 downregulates E2 ubiquitin-conjugating enzyme UBE2I (FC=-1.5) and E3 ubiquitin-ligase HUWE1 (FC=-2), both upregulated in ST group (FC=2.1 and 1.4, respectively). As ubiquitination is critical in inflammasome regulation and autophagy during infection, we tested CASP1 expression, finding a 1.5 FC downregulation in m215 and m215+ST groups ($p<0,05$). We also found CD44 (HCAM), an adhesion molecule involved in autophagy and highly expressed in the *Salmonella*-containing vacuole (SCV), as a target of miR-215. CD44 was upregulated (FC=1.5) during infection, and highly downregulated when miR-215 was transfected (FC=-4.4). Also, other proteins related to autophagy such as SCPEP1 (RISC), RAB2a and PTBP3 were found.

In conclusion, our results indicate that miR-215 plays an important role in the control of the host inflammasome activation and autophagy by targeting ubiquitination proteins, and can also impair the structure of the SCV and bacterial replication.

Keywords

microRNA, inflammation, salmonellosis, zoonosis, miR-215-5p

Genetic determinism of porcine plasma lipidome and its relationship to immunity

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Abstract

The modulation, activation and differentiation of several immune cells is highly dependent on lipid metabolism. The objective of this study was to analyse the genetic determinism of the porcine plasma lipidome and its association to animals' immune capacity. For this purpose, we quantified the plasma levels of 982 lipid molecules from a population of 300 healthy Duroc pigs. We estimated the heritability of the lipidome and their phenotypic and genetic correlations with 41 health-related traits. Additionally, genome wide associations studies (GWAS) were performed between the lipidome and 9,739,308 imputed single nucleotide polymorphisms.

Mean heritability estimates for the lipid species ranged from 0.04 to 0.91, with 186 of them obtaining mean estimates over 0.4. A total of 903 lipid species were found to be genetically associated with at least one of the health-related traits. GWAS analyses revealed 157,989 significant associations (adjusted $p < 0.05$) between 72,327 polymorphisms and 139 metabolites, identifying a total of 172 associated genomic regions. The strongest associations were found on chromosome 8 for the polymorphisms rs338500538, rs701893123 and rs338500538, which were associated with three lipid molecules. By overlapping the genomic regions associated to lipids with previously defined QTL for immunity traits, we revealed 22 regions associated to eight immunological traits. Within these genomic regions, relevant immunity modulators such as *ST3GAL1*, *SLA* and *IL2* genes were identified.

In conclusion, our results confirmed the genetic determinism of the blood lipidomic profile in pigs and highlight the relationship of lipid species with immunity and health-related phenotypes.

Keywords

Pig, Lipidome, Immunocompetence

Unveiling blood transcriptome regulatory variants involved in pig immunity

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Abstract

The transcriptional profile of peripheral blood in swine is known to reflect variations in immune functions. In the present work, we aimed to characterize transcriptional regulatory variants associated with innate and adaptative immune functions and health-related traits. Expression genome-wide associations studies were performed between the expression of 16,063 genes obtained from whole-blood RNA sequencing and 9,739,308 imputed single nucleotide polymorphisms in 255 healthy animals of a commercial Duroc line aged 60 ± 8 days.

A total of 12,386 expression quantitative trait loci (eQTLs) for 6,449 genes were identified. Comparison between eQTLs and genomic regions already described as associated with immunity traits revealed 358 eQTLs overlapping with 15 QTLs for 12 immunological traits. The highest number of overlapped regions were found for the relative abundance of T-helper cells and memory T-helper cells. Within these regions, immune-related genes such as members of the *CLEC*, *IGKV* and *KLR* families, *RBPJ*, and *ZAP70* were identified. Additionally, 605,069 genetic variants were associated with the expression of ten or more different genes and were considered as potential regulatory hotspots. Particularly noteworthy was the top hotspot variant rs3475331335, which regulates genes involved in immune-related biological processes such as cytokine-mediated signalling pathway (28 genes), positive regulation of response to external stimuli (25 genes) or myeloid cell differentiation (22 genes), among others.

In conclusion, this study reported regulatory variants and candidate genes associated with immune- and health-related traits in pigs, shedding new light on the regulatory mechanisms modulating pathways involved in immune functions.

Keywords

Pig, Transcriptome, Immunocompetence

Developing a cell based assay to study trained immunity in chicken NK cells

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Abstract

In mammals, memory NK cells have been described that show enhanced activation upon a secondary stimulation. This suggests trained immunity, memory formation of the innate immune system, in NK cells. Previous research demonstrated modulation of chicken NK cell activity, but whether this leads to trained immunity remains unclear. Therefore, the aim of the current study is to establish an *in vitro* assay to investigate trained immunity in chicken NK cells.

Primary chicken NK cells were cultured in medium, or medium supplemented with recombinant chicken IL-2 or IL-15 for 24 hours as primary stimulation. Next, cells were washed and after 2- 5 days of culture in medium, cells were harvested and restimulated with PMA/ Ionomycin or recombinant chicken IFN α . NK cell activation and viability were determined by flowcytometry. The delta NK activation upon restimulation was calculated and compared among different primary stimuli to assess NK training.

Culturing NK cells with IL-2 for 24 hours, followed by a 2-day rest before restimulation, yielded the highest percentage of CD107+ NK cells while preserving viability. Restimulation of NK cells primed with IL-2 resulted in a delta NK activation of $20.3 \pm 1.9\%$ which was higher compared to IL-15 primed cells ($7.9 \pm 0.05\%$) and unprimed cells ($10.3 \pm 1.0\%$).

In conclusion, we have developed an *in vitro* assay to measure trained immunity in chicken NK cells and are currently testing additional stimuli and readouts. In future studies this *in vitro* assay can be used to identify compounds able to induce trained immunity in chicken NK cells.

Keywords

trained immunity, natural killer cells, chicken

Modelling bovine tuberculosis infection in stem cell-derived macrophages

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Abstract

Bovine tuberculosis is a major disease with serious economic and social consequences. The pathogen responsible, *Mycobacterium bovis*, is inhaled into the lungs and engulfed by alveolar macrophages. The early macrophage-mycobacteria interactions are crucial in determining the outcome of infection; therefore, it is important to investigate these interactions using a reliable model. We have developed a system to study *M. bovis* infection using bovine embryonic stem cell-derived macrophages (bESC-dMs).

bESCs provide unlimited, experimentally tractable macrophages when put through a three-phase differentiation protocol which involves mesoderm induction, myeloid lineage commitment and macrophage maturation. Analysis by RT-qPCR shows that pluripotency gene expression decreases and macrophage gene expression increases during the differentiation process. Furthermore, flow cytometric analysis has demonstrated that mature bESC-dMs have high cell-surface levels of molecules involved in pathogen recognition and antigen presentation including CD163, CD86 and CD1b.

The bESC-dMs behave similarly to *ex vivo* bovine alveolar macrophages as both cell types are capable of phagocytosing fluorescent particles and both increase cytokine gene expression when exposed to LPS. We have visualised the presence of mycobacteria within the bESC-dMs using Rhodamine B-stained *M. bovis*. Flow cytometry of uninfected and *M. bovis*-infected bESC-dMs has demonstrated that the expression of CD80, CD206, and CD40 increase in response to infection.

ESCs can be genetically edited prior to differentiation, therefore, we have created IL10 knockout bESCs to study the importance of this immune regulator in *M. bovis*-infected macrophages. We have shown that bESC-dMs are a valuable tool to enhance our understanding of macrophage-pathogen interactions.

Keywords

Stem cells, Macrophages, Bovine tuberculosis

***Ascaris suum* induces systemic T follicular helper type 2 cells, but only a weak Th2 response in growing pigs**

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Abstract

Ascaris suum (*A. suum*) is a widespread zoonotic pathogen in swine. While humoral immune responses have been documented, little is known about the germinal center reaction leading to antibody production. Thus, we aimed to examine the role of T effector (TC) and T follicular helper (Tfh) cells during the hepatotracheal migration of *A. suum* in pigs.

The data for this study was collected from three independent experiments. Pigs were left untreated or orally inoculated with *A. suum* and dissected at different time points post-infection. Immunophenotyping was performed on systemic compartments and migration-affected lymphatic organs (liver, lung, mesenteric lymph nodes, and Peyer's Patches). The frequency of TC and Tfh cells, their transcription factor, and cytokine profiles were assessed.

Our data suggest that nematode infection does not increase Tfh cell frequencies. However, we found that pigs displayed a robust Tfh2-like phenotype, characterized by the expression of GATA3 and IL-4 following *A. suum* infection. Contrarily, systemic Th2 levels remained consistently low. Thus, we hypothesize that weak Tfh induction is an important feature of parasite infection and that the Tfh phenotype is crucial in forming a protective antibody response.

Pigs offer a unique opportunity to investigate the role of Tfh cells in infectious diseases. Our findings could enhance the in-depth characterization of Tfh cells targeting natural swine pathogens and might potentially explain the constant reinfections observed in *A. suum* infections.

Keywords

T follicular helper cells (Tfh), pig, *Ascaris suum*

Characterization of the immunostimulatory properties of a warthog microbiota component in porcine alveolar macrophages

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Abstract

The exploration of novel immunomodulatory components has been proposed to compensate the negative effects of antibiotic reduction by increasing animal resilience. Previous work from our group demonstrated that fecal microbiota transplantation from warthogs to domestic pigs induce partial protection from African swine fever (ASF) during experimental infection. In this direction, we isolated a bacterium from warthogs feces identified as *Rothia nasimurium* by 16s rRNA sequencing. This bacterium was selected for its ability to induce IFN-gamma secretion across different porcine cell types, thus suggesting a potential role as immunostimulator. Hence, the objective of this study was to characterize the immunomodulatory properties of this *Rothia nasimurium* isolate on porcine immune cells, and to evaluate its capacity to reduce ASF virus (ASFV) infection. Our results demonstrate that *Rothia nasimurium* alive can induce the secretion of several cytokines, including IFN-gamma and other pro-inflammatory cytokines like TNF-alpha, in different porcine immune cells. In addition, transcriptomic analysis showed that stimulation of alveolar macrophages with heat-inactivated bacteria robustly activated innate immune system pathways, including inflammasome-associated genes. Indeed, we further confirmed that inactivated *Rothia nasimurium* acts as a secondary stimulus for inflammasome activation, inducing the secretion of IL-1 β in LPS-primed macrophages. Finally, we demonstrate that activation of porcine macrophages with inactivated bacteria reduce their susceptibility to infection with different ASFV strains, and modify the corresponding innate immune responses. Altogether, these results suggest that *Rothia nasimurium* might be used as an immunostimulant to enhance animal resilience.

Keywords

Rothia nasimurium; immunostimulant; innate immunity.

The cytokine response to innate stimuli in blood of calves at birth and the occurrence of infectious diarrhoea

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Abstract

Newborn calves are susceptible to diarrhoea caused by infections. The capacity of calves to mobilize their innate immune defenses is key to explain the disease susceptibility at this early stage of life.

To phenotype this innate response, whole blood cells collected from 623 Holstein calves aged 1 to 2 weeks were exposed for 24h at 38.5°C to synthetic agonists mimicking exposure to pathogens (R848, LPS and MDP, ligands of TLR7/8, TLR4 and NOD2, respectively). The production of 15 cytokines and chemokines was quantified using Luminex technology.

A subset of cytokines and chemokines (CCL3, CCL4, IP10, IL-1RA, TNF α , IL-1 α , IL-1 β , IL-10 and IFN γ) had increased levels of expression after treatment of blood cells with agonists, R848 being the most potent. Using contingency approaches on the values of these cytokines between individuals/agonist treatments, we showed convergence between groups of strong responders for all conditions as well as for groups of weak responders. Nevertheless the distribution of individuals in these “high” versus “weak” categories varied with the agonist.

A multivariate redundancy analysis showed that there were significant relationships between the cytokines produced in the blood and the clinical status of the calves (symptoms, pathogens). However, the significant associations were dependent upon the agonist used to reveal the cytokine signature and the clinical parameter monitored.

To conclude, calves at birth show different abilities in their response to innate stimuli, which to some extent could explain the occurrence of intestinal infections. We are investigating the genetic control for the innate immune phenotype.

Keywords

calve, innate immunity, diarrhoea

Innate Cytokine Profile and the link to fecal microbiota around calving in dairy cows

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Abstract

Introduction: The microbiome is known to influence the immune system. Management of the transition period around calving may induce a shift in immune function and digestive tract microbiota in dairy cows.

Purpose: The aim was to investigate differences in the cytokine production profile to lipopolysaccharide around parturition in dairy cows and to evaluate the relationship with their fecal microbiota.

Methods: Blood and feces samples were collected from a cohort of 411 dairy cows from 25 different commercial farms before and after calving. Whole blood was stimulated with LPS to measure cytokine production using a 15-plex assay. Bacterial DNA from feces was sequenced based on the 16S rRNA gene. Clustering of dairy cows based on cytokine production was performed using Discriminant Analysis of Principal Components (DAPC). Analysis of Microbiome Composition using Bias Correction 2 (ANCOM-BC2) was then applied to the 16S RNA data and their relationship was further explored.

Results: Before and after calving, cytokine production with LPS stimulation was significantly different from the control condition. Clusters were identified with a difference in immune response and according to the calving. Regarding the microbiota, ANCOM-BC2 showed that the abundance of several taxa was affected by calving. Differences in microbiota taxa were also observed between cytokine-based clusters.

Conclusion: This study shows that calving affects the fecal microbiota of dairy cows. However, cows with different immune profiles after calving could also be distinguished by their fecal microbiota. Feces need to be further investigated as a potential health biomarker.

Keywords

microbiota, whole-blood assay, cattle

Clinical improvement of equine insect bite hypersensitivity lesion scores following immunotherapy with *Culicoides* recombinant allergens in combination with a TLR-4 agonist

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Abstract

Insect bite hypersensitivity (IBH) is an IgE-mediated allergic dermatitis of horses caused by *Culicoides* midges. Allergen immunotherapy (AIT) is the only disease-modifying treatment for type 1 allergies, but placebo-controlled studies showed a lack of efficacy of AIT using crude *Culicoides* whole body extracts. Consequently, *Culicoides* allergens have been identified and produced over the last decades.

The aim was to investigate in a placebo-controlled study if AIT using a pool of major *Culicoides* recombinant (r-)allergens could improve clinical signs of IBH.

Horses were scored monthly during the IBH season using a standardized clinical IBH lesion score. Horses were vaccinated subcutaneously at the beginning of spring, followed by 2 boost immunisations in the first and second treatment year. Eight horses received a placebo and 9 horses a pool of 9 *Culicoides* r-allergens, in aluminium and the TLR4 agonist MPLA as adjuvants. Allergen-specific IgE and IgG subclasses were determined by ELISA.

The IBH lesion score decreased between the pre-treatment and the 1st treatment year in both the AIT and placebo groups, but there was a significantly larger decrease of the average lesion score between the treatment and pre-treatment year in the AIT compared to the placebo group. The AIT effect was even more pronounced in the 2nd treatment year. After treatment significantly higher IgG4/7 levels were found in the AIT compared to the placebo group. This is the first placebo-controlled study showing a beneficial effect of AIT for treatment of IBH using pure *Culicoides* r-allergens.

Supported by Morris Animal Foundation grant no D20EQ-032

Keywords

Horse, Immunotherapy, Culicoides

A systems immunology approach reveals distinct roles of genetic and non-genetic factors in shaping variation of immune responses in cattle

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Abstract

One of the major challenges facing agriculture is to increase its production capacity to meet global demand while minimizing the reliance on synthetic inputs like antibiotics. These observations are particularly true for beef cattle breeding, notably Belgian White Blue (BWB) cattle which is the most muscular breed in the world. Historically, the selection process for these animals has prioritized production traits, often at the expense of health and disease resistance. However, recent studies in humans indicate that a significant portion of the variability of immune responses can be explained by genetic determinants. Applied to BWB, this indicates that there could be a considerable opportunity to increase the resistance of those animals to infectious diseases. Using systems immunology, we explored genetic and environmental factors driving immune variation in BWB. While immune variation was largely influenced by non-genetic factors such as seasonality, we identified ten loci with major effect on distinct immunophenotypes by genome-wide association studies. Moreover, a computational predictive model based on these genetic data was able to forecast cytokine responses to immune stimulations, offering new health management avenues. Taken together, our data have established a resource for understanding immune variability in ruminants that may pave the way to select animals with improved immunity. In the future, integrating such approaches across species could advance not just animal health but also our understanding of mammalian immune responses in general.

Keywords

systems immunology, cattle, genomics

Differential resistance to nematode infection is associated with the genotype- and age-dependent pace of intestinal T cell homing

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Abstract

The resistance of inbred mice to nematode infections varies depending on the extent of protective Th2 responses. The low resistance despite robust induction of GATA-3+T cells seen in C57BL/6 mice is partly due to the higher IFN- γ competent T-bet+Th2/1 present in GATA-3+ population of *Heligmosomoides bakeri* (*Hb*) infected C57BL/6 compared to BALB/c mice. We have shown that C57BL/6 mice, having fewer granulomas and higher worm burden compared to BALB/c, harbored a lot of these Th2/1 in the spleen and intestines. Furthermore, Th2/1 account for higher proportions in the GATA-3+ effector pool of mature compared to young mice, resulting in a decline of resistance to infection along host age.

We compared two mouse lines differing in resistance to infection with the enteric nematode *Hb* using cellular-based assays and flow cytometry.

Young infected BALB/c mice recruited higher frequencies of CD4+GATA-3+ type 2 cells and eosinophils to the small intestine within the first week of infection compared to age-matched C57BL/6 mice. This rapid Th2 recruitment correlated with extensive expression of CCR9 and higher frequencies of CD103+migDCs expressing ALDH+ in the gut-draining lymph nodes of the BALB/c line. However, older infected BALB/c mice displayed diminished ALD-activity and CCR9 expression, resulting in the delayed establishment of local type 2 responses.

In summary, the resistance of BALB/c compared to C57BL/6 against *Hb* infection correlates with rapid recruitment of effector cells to the infected gut, revealing the impact of age in efficient T cell response in the context of intestinal infections.

Keywords

Gut homing, T cell, age

Chicken 3D enteroids as tool to determine virulence of non-notifiable avian influenza

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Abstract

Avian influenza (AI) is a global problem, causing widespread harm to animals and public health. Low pathogenic AI (LPAI) strains cause mild or moderate symptoms while highly pathogenic AI (HPAI) strains cause up to 100% mortality in chickens. Moderately virulent LPAI strains, such as H3N1, H6N1, and H9N2, can induce 5-95% mortality. These moderately virulent LPAI viruses have become endemic in backyard and commercial poultry industry, making it necessary to review gaps in our understanding of these viruses at the host level. Typically, LPAI replicate in the respiratory tract. However, the virulent H3N1 exhibits broad tissue tropism and can replicate in the intestinal and urogenital tract. Chicken 3D enteroids comprised of multiple epithelial cell-types with an inside-out, media facing apical brush border conformation. Notably, chicken 3D enteroids have an inner core resembling the lamina propria, containing functional leukocytes. The aim is to examine chicken 3D enteroids as *in vitro* models to predict virulence of LPAI viruses. The infection of layer- or broiler-derived enteroids with virulent (H3N1), mildly virulent (H5N2, H6N1) and avirulent (H9N2) LPAI strains indicate differences in the H9N2 replication levels between genotypes. Differences in immune responses were observed between the lines, with immune responses dampen by H3N1 in layer-derived enteroids and increased in broiler-derived enteroids. The chicken 3D model has the potential for exploitation to study host-pathogen interaction and as a tool to determine the level of LPAI virulence, examine genotype-phenotype differences and tissue tropism.

Keywords

Chicken, avian influenza, 3D enteroids

Can we treat allergy with transgenic barley?

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Abstract

Insect bite hypersensitivity (IBH), an IgE-mediated allergy in horses, is caused by biting midges (*Culicoides* spp.). The responsible *Culicoides* spp. are not endemic in Iceland. However, exported Icelandic horses show a high IBH-prevalence. We developed special spiral bits to orally treat horses with transgenic barley expressing *Culicoides* allergen. The treatment induced an allergen-specific IgG response but was impractical. The aim is to develop a practical method to treat IBH-affected horses with transgenic barley expressing *Culicoides* allergen.

Healthy Icelandic horses were treated with barley mixture in stable buckets, 6 with Culo2p recombinant barley and 6 with control barley. In the initiation phase, week 0-8, fed 34 times with 7.5 g of recombinant Culo2p/horse. In the maintenance phase, week 26-30, fed 8 times with 1.75g rCulo2p/horse. Blood was collected bi-weekly and saliva two weeks post treatment. Culo2p-specific IgG and the IgE blocking capacity of the sera was analysed by ELISA.

Four out of six Culo2p barley treated horses produced Culo2p-specific IgG, particularly IgG1 and IgG4/7 which peaked at week 4, declined after week 8, and increased again in the maintenance phase. The induced IgG antibodies partly blocked IgE from Culo2p-binding. Culo2p-specific IgG4/7 was present in saliva of 4/6 horses.

Allergen-specific IgG immune response that partly inhibits IgE binding could be induced orally in horses by feeding them with transgenic barley. This can easily be done by the horse owners, but the approach needs to be verified for desensitization of IBH-affected horses against the respective allergen.

Keywords

Insect bite hypersensitivity, horses, oral AIT, barley expressed allergens

Virulent African swine fever virus infection of porcine monocytes leads to loss of ER function and subversion of SLA I

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Abstract

African swine fever virus (ASFV) is a large DNA virus of the *Asfarviridae* family that causes a fatal hemorrhagic disease in domestic pigs and wild boar. Infections with attenuated strains result in a milder clinical course and lower lethality. As target cells of ASFV, monocytes play a crucial role in T cell-mediated immune defense and pathogenesis.

By comparing the effect of the highly virulent "Armenia 2008" (ASFV-A) virus strain with that of the naturally attenuated "Estonia 2014" (ASFV-E) on cellular immune activation *in vivo* and on primary monocytes *ex vivo*, we asked whether ASFV-A infected pig monocytes are impaired for antigen presentation by viral immune subversion.

ASFV-A-infected monocytes are characterized by less swine leukocyte antigen class I (SLA I) on the cell surface than ASFV-E-infected monocytes. Despite the absence of a detectable reduction in steady-state SLA I mRNA/protein levels or effects on the presence of components of the antigen processing machinery, a marked decrease in maturation and surface presentation of SLA I was observed in ASFV-A-infected monocytes. The intracellular maturation block of SLA I was accompanied with a loss of functional ER structures and a pronounced formation of ER-associated aggresomes. This unresolved cellular stress affects overall host cell protein translation, mitochondrial function and induces caspase-3-mediated apoptosis. In contrast, no such cellular subversion phenomenon was found in ASFV-E-infected monocytes.

Our results suggest that in domestic pigs infected with the highly virulent ASFV-A, sequential subversion events occur in infected monocytes, leading to impaired T cell activation and downstream responses against ASFV.

Keywords

African swine fever virus (ASFV), pig, immune evasion

Identification and Characterisation of Porcine T Follicular Helper and T Follicular Regulatory Cells

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Abstract

CD4⁺ T follicular helper (Tfh) cells are critical for germinal centre (GC) formation, provide help to B cells for antibody class-switching and affinity maturation, and enable generation of GC-derived memory B cells and long-lived plasma cells essential for durable antibody production. In humans and mice, Tfh are characterised by expression of CXCR5, ICOS, PD-1, Bcl-6 and IL-21 while T follicular regulatory (Tfr) cells express FoxP3, Bcl-6, CXCR5 and PD-1, functioning as a regulatory counterpart to Tfh in GC reactions.

Increases in the frequency and/or activation of circulating Tfh correlate with vaccine-induced antibody responses; and expansion of dysfunctional Tfh during severe SARS-CoV-2 and HIV infections, together with increased frequencies of Tfr during chronic viral infections are associated with suboptimal antibody responses.

Few studies have examined the Tfh/Tfr cell dynamic during virus infections in species other than humans, non-human primates, or mice, in part, due to a lack of tools available to identify these cells. Here, we have designed a panel of antibodies for flow cytometry analysis composed of customised, cross-reactive, and novel reagents to characterise cells in porcine secondary lymphoid tissues and identify putative porcine Tfh cells as CD3⁺CD4⁺CD8 α ⁺CCR7⁺PD-1⁺Bcl-6⁺ICOS⁺CD27⁺c-Maf⁺ and Tfr cells as CD3⁺CD4⁺CD8 α ⁺CCR7⁺PD-1⁺Bcl-6⁺ICOS⁺CD27⁺CD25⁺FoxP3⁺. We are developing assays to assess the function of porcine Tfh and Tfr cells and employing spatial transcriptomics to gain insight into their roles in regulating B cell responses. These tools can be exploited to better understand GC responses in the pig and how these might be dysregulated during virus infections.

Keywords

Pig, Tfh cells, Tfr cells

Dissection of the antibody response to an antigen decorated VLP vaccine in rainbow trout

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Abstract

Virus-like particles (VLPs) based on recombinant viral proteins self-assembling into non-infectious particles which are structurally similar to the parental virus are highly immunogenic and have shown great potential as vaccines against several viral diseases in humans and husbandry animals including aquacultured fish. We recently showed that the decoration of VLPs with heterologous antigen from a bacterial fish pathogen using the SpyTag/SpyCatcher conjugation system could expand the use of VLPs as vaccines to include nonviral diseases. By vaccinating rainbow trout with VLPs decorated with the virulence associated surface protein A (VapA) from *Aeromonas salmonicida* we could thus induce generation of specific antibodies against *A. salmonicida* as well as protective immunity against the disease known as furunculosis. Ideally, the VapA antigen presented on the surface of the VLPs would be the key component recognized by the fish immune system. In order to determine whether the VLP carrier and/or the peptide-based conjugation component disturbed the antibody response, we here examined the induced antibody response against the individual vaccine components in ELISA. The results revealed that the fish produced antibodies to all vaccine components, and further that the relative distribution of titers depended on the origin of the VLP core as well as on whether the vaccine was emulsified with Freund's incomplete adjuvant or not. Selection of optimal VLP carrier as well as adjuvant thus represents key elements in the development of such vaccines for fish.

Keywords

Virus-like particles (VLPs), recombinant vaccine, antibody response

Comparative transcriptomic analysis of IAV infection in porcine organoids.

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Abstract

Introduction / Purpose

To understand how influenza strains emerge, evolve, infect and cause disease in animals and humans, better models are needed. This project will provide a three-dimensional (3D) cell system in which the regulation of genes in influenza infection are investigated, and the degree to which they determine productive infection and, therefore, disease. The results will lay the groundwork to the design of new and improved influenza vaccines, diagnostics and antiviral drugs to treat influenza infection.

Methods

We have established an *ex vivo* 3D organotypic air-liquid interface primary porcine respiratory epithelial cell culture system (ALI-PRECs) recreating a cell culture environment morphologically and functionally more representative of the epithelial lining of the swine trachea than traditional culture systems. Using these ALI-PREC models, our laboratory has investigated the molecular pathogenesis of human-, swine-, avian-lineage influenza A virus infection.

Results

Our studies demonstrated that these viruses trigger early innate immune events that disrupt the homeostasis of mucosal epithelia, including immune changes at physiological barriers, such as mucociliary responses, and changes at the molecular level, such as toll-like receptors, NOD-like receptors, and RIG-I-like receptors causing antiviral, cytokine, and chemokine activation leading to the recruitment of immune cells towards epithelial mucosa.

Discussion/Conclusion

We have established *ex vivo* culture models that mimic biologically and physiologically the original tissue/organ *in vivo*, thus contributing to the advancement of translational and biomedical research.

Keywords

Influenza A virus; comparative transcriptomics; porcine organoids

Protection against the obligatory intracellular parasite *Neospora caninum* is dependent on IFN- γ signalling in myeloid and endothelial cells

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Abstract

The protozoan *Neospora caninum* is a major cause of abortions in cattle and is responsible for large economic losses in dairy and meat industry worldwide. The host protective response to this obligatory intracellular parasite mostly relies on IFN- γ -dependent mechanisms, both in mice and in cattle. However, the target effector cells and the triggered mechanisms are not fully elucidated.

Here we aimed to evaluate the role of IFN- γ signalling in hematopoietic and non-hematopoietic cells for host protection against *N. caninum*. Bovine monocyte-derived macrophages infected with *N. caninum* and treated with IFN- γ elevated the expression of GTPases associated with autophagy and parasitophorous vacuole destruction. IFN- γ treatment resulted in increased parasite phagocytosis and death, as assessed by live imaging. Moreover, using IfngR1 conditional KO murine models, we found that protection was largely mediated by IFN- γ -dependent induction of parasite microbicidal mechanisms in myeloid cells, particularly in macrophages. Additionally, conditional KO mice in which endothelial cells could not respond to IFN- γ had significantly higher organ parasite burden than that observed in wild-type littermates. Single-Cell RNA-seq of infected conditional KO mice showed decreased expression of genes encoding immune related GTPases in endothelial cells, indicating a direct contribution of these cells to the host IFN- γ mediated protection. Altogether, our results implicate both myeloid and endothelial cells in the host resistance to neosporosis driven by IFN- γ .

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Keywords

Neospora caninum, IFN-gamma, endothelial cells

The use of precision-cut lung slices to assess *Mycoplasma hyopneumoniae* tissue interaction

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Abstract

Mycoplasma (M.) hyopneumoniae is the primary pathogen responsible for Enzootic Pneumonia (EP), a chronic respiratory disease affecting 30-80% of pigs worldwide. Based on the existing literature on other *Mycoplasma* spp., it was hypothesised that C-type lectin receptors (CTLRs) are interacting with *M. hyopneumoniae*, allowing for the pathogen uptake into cells, and therefore masking its recognition by the host's immune system. To assess the potential interaction, initially, the porcine genome was mined for the presence of CTLRs, using a homologue/orthologue approach with the murine/human and bovine genome. A total of 24 CTLRs expressed on antigen presenting cells were identified in the porcine genome, and of these, transcripts of a total of 16 CTLRs were confirmed by RT-PCR, and in a limited approach by qPCR, across multiple animals and lung lobes. In a second approach, the presence of sugar moieties within the *M. hyopneumoniae* wall was also investigated using genome screening approaches, lectin assays, and glycoprotein assessments. To further investigate the interaction of *M. hyopneumoniae* with porcine lung tissue, the precision cut lung slicing (PCLS) was successfully adapted to porcine lung tissue, and the aspects of the immune response of the slices assessed. PCLS were successfully established under serum-containing and serum-free conditions. Exposure of PCLS with *M. hyopneumoniae* resulted in pronounced differences in the immune response generated. *M. hyopneumoniae* strain 232 induced an IL-1b response 24 hours post-infection (hpi), whereas a TNF- α response was not detected within the first 48hpi.

Keywords

precision cut tissue slices; *Mycoplasma hyopneumoniae*, C-type lectin receptors

Chicken intestinal organoids: a novel method to measure the mode of action of feed additives

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Abstract

The paucity of physiologically relevant models has limited research of poultry gut health and led to an over-reliance on the use of live birds for experiments. The development of 3D intestinal organoids created many opportunities and a major advantage of floating organoids is the combination of a complex cell system with an easily accessible apical-out orientation grown in a simple culture medium without an extracellular matrix. Here we investigated the impact of organic acids and essential oils on the innate immune responses and kinome of chicken intestinal organoids in a *Salmonella* challenge model. To mimic the *in vivo* exposure of the intestine to the product, the organoids and *Salmonella* were pre-treated with OA+EO and organoids were either uninfected or infected with *Salmonella* and bacterial load in the organoids was quantified. The treatment of the organoids with OA+EO resulted in a significant decrease in the bacterial load compared to controls. The expression of 88 innate genes was investigated using a qPCR array. *Salmonella* invasion of the untreated intestinal organoids resulted in a significant inflammatory response and intracellular signalling. In contrast, the inflammatory responses in OA+EO treated-organoids challenged with *Salmonella* were significantly downregulated. The kinome array data suggested decreased phosphorylation elicited by the OA+EO with *Salmonella* in agreement with the gene expression data sets. This study demonstrates that the *in vitro* chicken intestinal organoids are a new tool to measure the effect of feed additives in a bacterial challenge model by measuring innate immune and protein kinases responses.

Keywords

organoids; emerging technologies; immune models

Expanding the diagnostic toolbox in aquaculture: A case study of hemorrhagic smolt syndrome demonstrates the value of blood biochemistry analysis in farmed salmon.

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Abstract

The Norwegian aquaculture industry is lacking fast and non-invasive approaches for decision making during critical phases of production, such as before stressful handling operations of fish. Biochemical blood analysis is an important tool in human – and veterinary diagnostics, and a small non-invasive blood sample can be highly informative and with high prognostic accuracy. In aquaculture however, blood biochemistry is not routinely used for diagnostics. The aim of the “Indicator” project is to validate the use of blood biochemistry, including candidate blood biomarkers in aquaculture practices.

Non-infectious production-related diseases have become an increasing health and welfare issue during the intensive freshwater phase of Atlantic salmon production, and can also affect farmed fish into the seawater phase. A case study of hemorrhagic smolt syndrome (HSS) exemplifies the value of using blood biochemistry analysis in farmed salmonids. Our results show a significant reduction of blood protein, elevated blood metabolites and electrolyte disturbances, especially a significant decrease of blood calcium levels. Metabolic hormones T4 and T3 were significantly decreased in HSS fish, whereas the stress hormone cortisol was markedly elevated. Interestingly, preliminary data indicate increased blood clotting time in HSS affected fish, suggesting disturbances in blood coagulation.

Our results show the potential of blood biochemistry analysis for assessing health and welfare of farmed fish. Such analyses would be a highly valuable addition to the limited diagnostic aquaculture toolbox.

Keywords

Aquaculture, blood biochemistry, fish welfare

Identification of deleterious genetic variants in the bovine transcriptome from Holstein cattle naturally infected with *Mycobacterium avium* subsp. *paratuberculosis*

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Abstract

Previous genome-wide association (GWAS) studies have identified single nucleotide polymorphisms (SNPs) associated with bovine paratuberculosis (PTB) susceptibility and resistance. However, most of these SNPs are located in non-coding regions, which makes functional interpretations challenging. This study used RNA-Seq data from blood and ileocecal valve samples from control cows without lesions (N = 4), and with focal (N = 5) and diffuse (N = 5) PTB-associated lesions in gut tissues to identify fixed functional SNPs in coding regions with deleterious effects in the corresponding coding proteins and unique to each group of animals. The RNA-Seq reads were aligned against the bovine ARS-UCD1.2.109 reference genome using the *STAR* aligner. SNPs were called using *BCFtools*. Variant effect prediction and functional analyses using the identified candidate genes were performed with *VeP* and *ClusterProfiler*, respectively. From the 856, 625, and 603 SNPs specifically identified in the transcriptome of cows without lesions and with focal and diffuse lesions; 31, 15, and 31 variants had deleterious effects, respectively. Pathways enrichment analysis using the deleterious SNPs-associated positional candidate genes revealed an enrichment in genes involved in the regulation of apoptosis and lipid biosynthesis in cows with focal lesions and in antigen recognition and presentation in cows with diffuse lesions and without lesions. Our results provide insight into the underlying genetic architecture of PTB and might be useful in breeding strategies to improve PTB resistance in dairy cattle.

Keywords

deleterious genetic variants, paratuberculosis, cattle

Muramyl di-peptide (MDP) is a potent inducer of “trained immunity” in porcine monocytic cells

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Abstract

Innate immune memory refers to long-lasting functional adaptations in innate immunity from a brief encounter with stimuli, achieved through epigenetic and metabolic changes with two opposing outcomes termed “training” and “tolerance”.

Here, we exploited time-course bulk RNA-seq, ATAC-seq, functional assays and pharmaceutical inhibitors to explore the training effects of MDP and LPS on porcine monocytic cells.

Priming of porcine monocytes with MDP led to a mild inflammatory response compared to LPS which was characterized by enhanced expression of IL-1A, prostaglandins and enrichment for responses mediated by the NFkB family of transcription factors. Following removal of stimuli and resting of the cells for 6 days, MDP-primed cells showed a distinct transcriptional and epigenetic signature compared to LPS as well as to unprimed controls. MDP-primed cells had increased expression of genes related to metabolism, defence, antigen processing and presentation during rest phase. Gene set enrichment analysis indicated prominent enrichment for responses mediated by distinct transcription factors. For MDP, these were linked to IRF and NFkB, while for LPS E2F responses were prominent. Chromatin accessibility was aligned with the gene expression pattern for several genes and their upstream transcriptional factors. The altered transcriptional and epigenetic landscape of MDP-primed cells resulted in functional changes upon second stimulation with LPS. While LPS-primed cells showed reduced production of pro-inflammatory cytokines reminiscing endotoxin tolerance, MDP-primed macrophages showed enhanced production of TNF, IL-1 β and IL-6 reflecting a trained phenotype.

Altogether, our results indicate that MDP might be a promising candidate for inducing trained immunity in porcine monocytic cells.

Keywords

MDP, trained immunity, tolerance

Study of bovine immunopathogenetic pathways during *Mycobacterium avium* *subsp. paratuberculosis* infection in Marchigiana beef cattle a native Italian breed

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Abstract

Bovine Paratuberculosis is a chronic enteritis caused by *Mycobacterium avium* *ssp. paratuberculosis* (MAP). Infection of calves occurs early via fecal-oral route but the clinical signs of Paratuberculosis do not appear always and anyway not before 2-3 years of age.

In the early stages of infection, the host innate and cell-mediated immunity are essential to contrast MAP infection or to contain Paratuberculosis progression, and thus it is related to Paratuberculosis susceptibility/resistance (S/R).

In the different stages of Paratuberculosis, MAP immune cells interaction induces expression of pro or anti - inflammatory cytokines and peripheral blood cells show different cytokines profiles.

Through a longitudinal study, in a MAP-infected herd of a native Italian beef breed, *Marchigiana* cattle breed, IFN- γ tests, ELISA, qPCR, and cultures were performed to evaluate the progression of MAP infection. In particular, twelve subjects were categorized in three phenotypic groups: healthy uninfected; healthy but MAP-infected (IFN- γ test positive); Paratuberculosis affected cattle (ELISA, qPCR, culture positive).

Gene expression and secretion of interleukins, were evaluated by RT-qPCR and *ELI-Microarray* using whole blood post-stimulation with Avian and Johnin PPD. After PPDs stimulation, infected and affected animals showed a significant up-regulation ($p < 0.05$) of *IL1A*, *IL18* and *IFN γ* genes, compared to healthy ones; in the same groups significantly higher levels of IL1 β , IL6 and IP10 (proteins), were detected.

This approach could help to characterize cattle breed carriers of Paratuberculosis resistance genetic traits and also contributes to identify possible new biomarkers of the disease.

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Keywords

Paratuberculosis, cytokines, gene expression

Immune traits are affected by genetic selection for faecal enterotypes in pigs

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Abstract

The faecal microbiota of 60-day-old Large White pigs reared in same conditions can be structured into two enterotypes, for which the keystone genera are *Prevotella* (*P*) and *Mitsuokella* (*M*) or *Ruminococcus* (*R*) and *Treponema* (*T*). We generated two pig lines HPM and HRT selected for the relative abundance of either *P* and *M* or *R* and *T*, respectively. Each line showed an increase in the prevalence of the selected enterotype over three successive generations. We used 40 animals per line from the 3rd generation to investigate if at 60 days of age, they display differences in the microbiota of the small and large intestines, and in immune traits. Using 16S gene sequencing, the two lines were shown to harbour strong microbiota differences at the descending colon (648/1334 ASV and 79/144 genera differentially abundant), with less differences at the ileal Peyer's patches (38/435 ASV and 18/126 genera). Piglets from the HPM line exhibited a higher number of eosinophils and natural IgM, while piglets from the HRT line showed higher counts of CD4⁺ CD8⁺ T cells and stronger phagocytosis activity. Overall, our results show that direct genetic selection for the composition of the faecal microbiota is not only associated with changes in the microbiota of other sections of the gut, but also with host traits related to immunity. Those divergent pig lines are therefore a powerful tool for better understanding the combined effects of host genetics and gut microbiota on phenotypes relevant to sustainable livestock systems, including health and immune traits.

Keywords

microbiota, genetics, immune traits

Design of a Porcine ETEC Multi Epitope Fusion Antigen vaccine

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Abstract

Weaning affect piglet's health enormously with its changes in diet, social and environmental life conditions. Going from sows' milk to solid feed influence the microbial-, mucosal- and immunological-barriers of the intestine and result in loss of bacterial diversity in the gut and increased risk of Enterotoxigenic *Escherichia coli* (ETEC) infections leading to post weaning diarrhea (PWD). With an aim to vaccinate and broadly neutralize ETEC, we have targeted both transmissible plasmid-encoded and highly conserved chromosomally encoded pathogen specific virulence factors from porcine ETEC strains in a Porcine ETEC (POETEC) multiepitope fusion antigen (MEFA) design process. Initial immunization studies in mice and neonatal pigs with a toxoid version of the human *E. coli* heat-labile enterotoxin LT with epitopes from a porcine ETEC strain embedded in the LTa subunit (ZETEC20), revealed species differences with low ZETEC20 immunogenicity in pigs. This prompted us to design a simple POETEC5 construct with only duplications of selected ETEC epitopes separated by GGS linkers. Pig immunization studies with POETEC5 showed increased immunogenicity compared to ZETEC20 and with single peptide-specific serum IgG responses to four of the ten peptides included. In an attempt to improve the response even further, we increased the number of epitope repeats to four, which did not increase immunogenicity. We also added the bacterial FimH adhesion molecule to our antigen (POETEC9), which did not interfere with immunogenicity of previous epitopes.

Here we show humoral immunogenicity of a multiepitope fusion protein simply designed as porcine ETEC epitope repeats with GGS linkers.

Keywords

ETEC, Antigen design, Vaccine

Veterinary Toolkit. Isotype cross reactivity of anti-porcine IgG and IgA reagents

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Abstract

The market for veterinary immunology reagents is small and it is a fear that not all reagents are pre-market scrutinized as vigorously as they should. Here, we investigated the specificity of commercially available polyclonal goat anti-pig-IgG and -IgA HRP conjugated antibodies in ELISA. Reactivity was assessed against the pb27 mAb, which was recombinantly expressed as isotypes IgG1, IgG2a, IgG2b (IgG4a), IgG2c (IgG6.1), IgG3, IgG4 (IgG6.2), IgG5b (IgG5.1), IgG5c (IgG5.2) and dimeric IgA (dIgA). The purified antibodies were coated onto ELISA plates at 1 µg/mL in a 2-fold dilution, and reacted with the different HRP-conjugated secondary pAbs or anti-porcine IgA (clone K61 1B4) biotinylated monoclonal antibody followed by Streptavidin-HRP.

The anti-IgG pAbs from Biorad and Bethyl showed similar high reactivity with all IgG isotypes and cross-reacted with dIgA. Although dIgA reactivity was lower than for any IgG isotype, the IgG1 response was only ca 4-fold higher than dIgA, while the IgG2b response was about 8-fold higher than IgG1.

The anti-IgA pAb from Biorad clearly cross-reacted with IgG with around 30-60 fold higher reactivity to dIgA than IgG isotypes. In contrast, the anti-IgA pAb from Bethyl was similar to mAb K61 1B4 with more than 1000-fold difference in reactivity between dIgA and any IgG isotype.

As the concentration of IgG is much higher than IgA in serum, the Biorad pAb anti-IgA in reality measures serum IgG when used in serum samples.

Keywords

antibody cross-reactivity, ELISA, Toolkit

20-color flow cytometry in a veterinary species – yes, we can!

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Abstract

The pig is an important farm animal and in-depth understanding of the porcine immune system is crucial for setting the path towards improved vaccine development and overall health status of pigs. Flow cytometry (FCM) is an important tool to study phenotypical and functional aspects of immune cells in detail. During the last years progress was made in designing and validation of more complex multi-color FCM panels. This firstly enables to investigate various subtypes of immune cells in a single sample, and secondly reduces sample number per animal and study, thus supporting the 3Rs. Here we report the analysis of a 20-parameter panel (19 markers + viability dye) on porcine PBMCs using a combination of species-specific monoclonal antibodies (mAbs), that were either commercially available or in-house conjugated, and anti-human/mouse cross-reactive mAbs. Special emphasis was put on the validation of antibody reagents, e.g. titration to define the optimal staining index or confirmation of cross-reactivity of mAbs. Samples were measured on a full-spectrum cytometer equipped with three-lasers (Cytex Aurora analyzer, 405nm, 488nm, 640nm). To analyze this multi-dimensional data, t-distributed stochastic neighbor embedding (t-SNE) was used. Within PBMCs, we could not only define major lymphocyte subsets like B, T and Natural Killer cells, but also characterize defined lymphocyte subsets like distinct T-cell populations ($\alpha\beta$ and $\gamma\delta$), as well as defined differentiation or activation stages (naïve, central, and effector memory) in one sample. Likewise, more rare subsets like plasmacytoid dendritic cells and non-conventional NKT cells could be clearly depicted.

Keywords

flow cytometry, multi-color, swine

Potent interferon-alpha responses against ASFV by plasmacytoid dendritic cells are STING pathway dependent and require tight junctions with infected macrophages

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Abstract

While several African swine fever virus (ASFV)-encoded proteins potentially interfere with the cGAS-STING pathway at different levels to suppress interferon (IFN) type I production within the infected macrophage, systemic IFN- α is induced in the early stages of ASFV infection in pigs. The current work presents a pathway how such responses can be induced, at least *in vitro*. We demonstrate that infection of monocyte-derived macrophages (MDMs) by ASFV genotype 2 strains is highly effective but immunologically silent, as neither IFN- α , IFN responsive genes nor TNF was detected upon infection. Moreover, ASFV also failed to directly activate plasmacytoid dendritic cells (pDC). However, co-culture of pDCs with ASFV-infected MDMs, led to a robust pDC response characterized by high levels of IFN- α and TNF. Similar to other viruses pDC responses by infected macrophages required integrin-mediated cognate interactions with ASFV-infected MDM. Inhibitor studies indicated that the STING pathway and formation of tight junctions is required to activate pDC. We also found that pDC responses were enhanced with IFN- γ -polarized macrophages and also by IFN- γ -pretreatment of the pDC, highlighting the impact of the immunological microenvironment on the interaction between pDC and macrophages. These findings suggest that the IFN- α detected during ASFV infection in pigs could result from cognate interaction of pDC with ASFV-infected macrophages.

Keywords

plasmacytoid dendritic cells, ASFV, interferon

Characterization of a novel marker staining memory subsets in porcine $\alpha\beta$ T cells in age- and antigen-specific context

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Abstract

Memory formation is an important feature of the adaptive immune system, pivotal for managing various infectious diseases. $\alpha\beta$ T cells can be divided into naïve, central memory (CM), and effector memory (EM) subsets. A recently identified monoclonal antibody (mAb), clone 6C5 2H4, selectively stains memory subsets of porcine $\alpha\beta$ T cells, excluding naïve T cells. Using full-spectrum flow cytometry, we found the marker expressed on CD4 T cells, specifically on CD8 α ⁺CD27⁺ CM and CD8 α ⁺CD27⁻ EM subsets in blood, lymph node, spleen, and lung, except for CM in the blood. Likewise, on CD8 T cells, for all organs the marker was absent on CD11a^{dim}CD27⁺ CM, while being expressed on CD11a^{high}CD27^{-/dim} early and late EM cells. Additionally, in blood, we noted an age-related increase in lymphocytes stained with our marker (aged 8 to 181 weeks). Notably, at about 1-year of age, the subset of CD4 CM recognized by our mAb peaked before declining, while for CD4 and CD8 EM this subset plateaued to remain at high levels.

Upon *in vitro* restimulation with PCV2 vaccine-specific antigen, we observed production of IFN- γ , TNF- α , IL-17A and IL-2 of CD4 and CD8 CM and EM subsets identified by our novel marker. Notably, CM cells stained with our marker tended to produce more cytokines, while EM cells showed the opposite. Despite these findings, the specific antigen recognized by the novel mAb remains elusive, with ongoing identification efforts. Future research on this memory subset promises to enhance understanding of antigen-specific immune responses in pigs.

Keywords

memory marker, T cells, swine

The TRDC-knockout pigs lacking $\gamma\delta$ T cells: an update

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Abstract

The tripartite organization of the lymphocyte compartment, consisting of three subsets of lymphocytes: B cells, T cells expressing the $\alpha\beta$ T cell receptor (TCR), and T cells expressing $\gamma\delta$ TCRs, appears to be fundamental as it evolved independently in jawed and jawless vertebrates. An important variation between species is the quantity and the receptor variability of circulating $\gamma\delta$ T cells. In contrast to mice, pigs belong to the $\gamma\delta$ T cell high species providing new opportunities to study functional aspects of these enigmatic immune cells at the molecular level. Recently, we have established TRDC-knockout pigs by intracytoplasmic microinjection and somatic cell nuclear transfer resulting in healthy living $\gamma\delta$ T cell deficient pigs while heterozygous animals had about 50 % of $\gamma\delta$ T cells compared to wild type (wt) pigs. Under normal housing conditions no enhanced susceptibility to infections was observed for both TRDC-KO and heterozygous male and female pigs up to 3 years of age. The composition of remaining leucocyte subpopulations seems to be unaffected by the reduction (heterozygous) or depletion (KO) of $\gamma\delta$ T cells. Genome-wide transcriptome analyses in PBMC revealed a pattern of changes reflecting the impairment of known or expected $\gamma\delta$ T cell dependent pathways. Histopathology did not reveal developmental abnormalities of secondary lymphoid tissues. Nevertheless, challenging the TRDC-KO pigs with an attenuated virus revealed that TRDC-KO pigs generated a significantly lower neutralizing antibody titer as the syngeneic controls, indicating a reduced immunoreactivity.

Keywords

GD T cells, porcine, TRDC

How to talk to the mother's immune system? Lessons from the foal.

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Abstract

In early equine pregnancy, a highly invasive trophoblast cell subpopulation, the chorionic girdle cells, invade the endometrium and form endometrial cups (EC). These cells express classical MHC molecules, thereby stimulating a humoral and cellular immune response, resulting in a massive accumulation of maternal CD4⁺ and CD8⁺ T cells around the EC. Nevertheless, no immediate destruction of endometrial cups by maternal lymphoid cells occurs, presumably due to immune tolerance. Although the environment of EC is rich in TGFB and in FOXP3⁺, CD4⁺ T cells, the mechanisms leading to tolerance have not been elucidated. Recently, we discovered that equine trophoblast cells secrete pregnancy-specific glycoproteins (PSGs). Since human and murine PSGs activate latent TGFB, we hypothesized that equine PSGs may have a similar activity. We performed plasmon surface resonance experiments to show that equine PSG CEACAM49 can directly bind to the latency-associated peptide (LAP) of both TGFB1 and TGFB2. We then found that the binding of CEACAM49 leads to the activation of TGFB1 as determined by both ELISA and cell-based assays. Based on our results, we hypothesize that activation of latent TGFB in the EC environment by equine PSGs secreted by invasive trophoblast cells, could contribute to the generation of regulatory T cells (Tregs) to maintain immune tolerance.

Keywords

Equine, Tregs, Embryo

What's in your toolbox? Immune reagent development for veterinary species

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Abstract

The ability to deeply interrogate immune status has been crucial to advancements in infectious disease and vaccine research. Flow cytometry and monoclonal antibody technology revolutionized the ability to assess immune cell status and function. Starting in the 1990's, various veterinary immunologists organized and established many of the antibody clones available for livestock species today. Workshops attempted to validate reagent specificity and standardize nomenclature. More than thirty years later, rapid advances in technology and disproportionate funding have limited reagent availability for veterinary species when compared to rodent and human counterparts. While efforts by the veterinary immunology community have contributed to building the toolbox, groups have fractured and re-established over the decades, with roadblocks and gaps often reassessed. Adapting to animal welfare regulatory changes, limited funding, and minimal commercialization options will continue to challenge expansion of the veterinary immunology toolkit. Assessment of cross-reactivity of already available commercial or generated antibodies to multiple species warrants consideration, as well as incorporation of new technologies for antibody development. In addition, effective communication across the veterinary immunology research community is necessary to synergize efforts to expand the toolbox, but also limit unnecessary duplication. What tool will you bring?

Keywords

Toolbox

Innate Immune Gene Conservation and Diversity : Relevance to One Health

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Abstract

Animal innate immune systems are coded for by genes which have been highly conserved over the course of evolution; they are complemented by genes which have been more recently expanded in number and which have acquired new functional roles. From the discovery of a novel avian -specific TLR gene and multiple clusters of host defence peptide (HDP) genes across multiple livestock species, gene discovery in the pre-genome era led to a new insights into the structure and function of the innate immune system across species. These offer new opportunities for controlling infections via therapeutics and breeding strategies to enhance animal health. Moreover, our research has illustrated the interconnectedness of immune components between species and across physiological systems with β -Defensins now known to play critical roles in fertility. Our latest insights implicate these HDPs in the regulation of the microbiome and also for anti-fungal immunity. Importantly, with conserved pathways controlling innate resistance to infection comparative immunology approaches are informing research into human and livestock health and disease prevention, particularly in an era of mounting antimicrobial resistance and pandemic risk.

Keywords

Innate, defensins, TLRs

Epitogen: Transformative Platform For Veterinary Diagnostics and Vaccines Development

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Abstract

Timely diagnosis and efficient affordable vaccines are essential for effective prevention, management, and eradication of diseases within veterinary medicine. EpitogenX introduces two innovative technologies, EpitoPredikt and EpitoGen, designed to address persistent diagnostic and prophylactic challenges in the veterinary sector. EpitoPredikt is a software tool developed utilising proprietary data, capable of precisely identifying immunodominant epitopes for B-cells and potentially T-cells, along with the corresponding antibody response (IgG, IgM). The second technology, EpitoGen, is a protein-scaffold bioengineered to accommodate peptides and facilitate their expression in native conformation. The scaffold enables seamless integration of peptides/subunits, having a multiplexing capacity of up to 50 peptides ranging from 5 to 1000 amino acids. This versatility facilitates the development of precise serology tests and potent peptide-based vaccines through the creation of custom chimeric complexes. With high yield (>200mg/ml) and stability, the resulting chimeric antigen makes the technology affordable with quick turn-around time. EpitoPredikt and EpitoGen together represent transformative tools, promising fast development of accurate and efficacious diagnostic assays and vaccines, with the potential transforming the field of veterinary medicine.

Keywords

EpitoPredikt, EpitoGen, Multiplexing,

Contribution of red blood cells to the antiviral immune response against Piscine orthoreovirus; the causative agent of heart and skeletal muscle inflammation in Atlantic salmon

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Abstract

The nucleated red blood cells of fish play an important role in sensing systemic pathogens. A prevalent viral disease in farmed Atlantic salmon is heart and skeletal muscle inflammation (HSMI), caused by *Piscine orthoreovirus* (PRV) genotype 1. Other diseases caused by PRV are erythrocyte inclusion body syndrome in farmed Coho salmon (caused by PRV-2), and HSMI-like disease with anemia in farmed rainbow trout (caused by PRV-3). These diseases are all characterized by initial replication of PRV in red blood cells, and this study aims to clarify the role of red blood cells in the disease pathogenesis.

Through controlled PRV challenge trials, primary red blood cell culture experiments and transcriptome analyses, we have explored red blood cell gene expression in response to PRV in Atlantic salmon. We have also compared the differential gene expression in response to PRV-1, Inactivated PRV-1, PRV-2 and PRV-3, and discuss the putative link between transcriptome responses, pathological consequences and cross-protection.

This study shows that salmonid red blood cells express genes associated with antigen processing and presentation, T-cell interacting cytokines, chemokine receptors, and a wide range of antiviral effectors. A difference in interferon response factor expression between red blood cells and two other salmonid cell lines may be associated with viral susceptibility. The timing of antiviral responsiveness and differential expression of genes involved in lysosomal and signaling/transcriptional mechanisms characterize the response to pathological vs non-pathological PRV genotypes. The results provide new information on the link between Atlantic salmon red blood cell responses and disease development.

Keywords

Atlantic salmon, Piscine orthoreovirus, red blood cell

IgG heavy chain glycosylation in Holstein-Friesian calves aged from one to four months

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Abstract

Introduction: It is known that expression of terminal monosaccharides of IgG heavy chains' glycans determinate pro-or anti-inflammatory phenotypes of this immunoglobulin class. In this study we analysed age-related changes in the expression of terminal galactose and sialic acid on IgG heavy chains in calves aged from one month (starting pre-ruminant to the ruminant phase transition; almost no maternal IgG) to four months (ruminant digestion established; IgG synthesis established).

Methods: The IgG was isolated by protein G affinity chromatography from pooled peripheral blood serum samples of calves aged 1, 2, 3, and 4 months (20 calves per group). The expression of galactose and sialic acid on IgG heavy chains was analysed by *Ricinus communis* (RCA I) and *Sambucus nigra* (SNA) lectin blotting.

Results: The expression of galactose on IgG heavy chains was highest in one-month-old calves, decreased gradually with age, and in fourth-month old calves dropped to 40% of the initial value. The expression of sialic acid was lowest in one-month-old calves, then sharply increased three times in two-month-old calves and stayed almost unchanged till four months of age.

Discussion: Observed age-dependant increase the sialic acid expression on IgG heavy chains from the first to the fourth month of life indicate an age-related increase in IgG molecules having anti-inflammatory phenotype. This might represent mechanisms of protection of immature tissues from inflammatory damages in calves. If the increased level of sialic acid could be associated with the pre-ruminant to ruminant digestion transition and adaptation to rumen microbiome remains to be determined.

Keywords

calf; immunoglobulin; sialic acid.

In vitro characterisation of a genotype I African swine fever virus with genomic deletion isolated from Sardinian wild boar

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Abstract

African swine fever virus (ASFV) causes a devastating disease affecting domestic and wild pigs. A remarkable genetic stability of Sardinian ASFV isolates was described between 1978 and 2018, nevertheless in 2019 isolates with a sustained genomic deletion (4342 base pairs) were identified in wild boars. In this study, *in vitro* experiments with monocyte-derived macrophages (moMΦ) were carried out to unravel the phenotypic characteristics of this deleted variant.

Cells were infected with the deleted 7303WB/19 or the virulent Sardinian 26544/OG10 (both genotype I), alongside mock-infected controls, and virus-cell interaction was investigated with a vast array of techniques.

7303WB/19 presented a lower growth kinetic in moMΦ compared to 26544/OG10, using either a high (1) or a low (0.01) multiplicity of infection (MOI). In agreement, flow cytometric analysis showed that 7303WB/19 presented lower intracellular levels of ASFV proteins. Then, we assessed the release of key cytokines and expression of 84 antiviral defence genes in response to infection (MOI = 1). We observed no differences in the cytokine profile of macrophages infected with either ASFV strains. Gene expression data showed that both viruses presented a similar pattern, with up-regulation of *DHX58*, *DDX58*, *IFIH1*, *IRF7*, *ISG15*, *MIX1*, and *OAS2* at both 3 and 21 hour post-infection (hpi). At 21 hpi, both viruses triggered up-regulation of *CCL5*, *CXCL10*, *CXCL11*, but with statistical significance only for 7303WB/19.

Overall, these data suggested that the deleted 7303WB/19 possesses an attenuated phenotype. *In vivo* studies should be performed to fully characterise the phenotype of this deleted viral variant.

Keywords

ASFV, macrophages, growth defect, cytokines, anti-viral genes

Equine intestinal mucosal 'kill zone': characterization of the mucosal barrier of the small and large intestines and its reflection in feces.

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Abstract

Introduction: The intestinal mucosal 'kill zone' consists of the cooperation between secretory immunoglobulin A (SIgA) and components of the microbiota that coexist in the intestinal mucus layer. The purpose of this study was to determine the composition of the 'kill zone' in different parts of the digestive tract of nine healthy horses (small intestine, cecum, and colon) and its reflection in faeces.

Method: Samples of fecal, intestinal contents and biopsy specimens were collected from nine healthy slaughtered horses. We determined the composition of the microbiome (next generation sequencing of the 16S rRNA gene analysis), the amount of SIgA content (ELISA), and the number of SIgA producing cells (Immunohistochemical analysis).

Results: The diversity of the microbiome (according to Faith's PD, Pielou's evenness and Shannon index) was highest in the luminal contents of the colon ($p < 0.001$). The highest amount of SIgA in the contents was found in the small intestine ($p < 0.001$), and the highest number of IgA+ cells was found in the caecum ($p < 0.05$). No significant differences were found between the colon and the stool samples.

Discussion: The study showed that the composition of the mucosal kill zone differs significantly between the small and large intestine. Furthermore, the caecum was identified as a separate compartment that showed a high capacity for SIgA production. The fecal sample reflects the current state of the large colon. An increase in the abundance of the *Proteobacteria* in feces may be the sign of damage of the mucosal barrier, suggesting the presence of intestinal disorders and dysbiosis.

Keywords

microbiome, intestine, secretory immunoglobulin A,

Vaccination with a *Lawsonia intracellularis* subunit vaccine mitigated some disease parameters but failed to affect shedding

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Abstract

Lawsonia intracellularis (LI) is an economically important bacterium that is the causative agent of ileitis in pigs. In developing a subunit vaccine, we have focused on different immunization routes and adjuvants. This has led us to identify two formulations for further evaluation that may prove to be efficacious in protecting against disease.

A challenge trial was done to measure immunogenicity and protection. Group 1 and Group 2 piglets (n=20) were vaccinated with three recombinant LI proteins; F, G, and Y, with two adjuvants; A1 or A2. Group 3 piglets (n=20) were administered saline. Vaccination occurred at five and eight weeks of age. At eleven weeks all animals were challenged with LI. To measure immunogenicity, serum and mucosal antibody responses were assessed. To determine protection, average daily gain (ADG), bacterial shedding in feces and gross lesions were evaluated.

Both vaccines induced significant systemic humoral immune responses after vaccination but weak mucosal responses. Importantly, Group 2 animals had better ADG than those in Group 3. Group 1 animals had significantly lower quantities of the pathogen in feces than any other group. Group 2 animals had significantly lower gross lesion scores and gross lesion lengths than other groups.

Currently, there is no subunit vaccine for *L. intracellularis*. Here, vaccination with a subunit *L. intracellularis* vaccine comprised of three recombinant antigens determined that the vaccine was immunogenic. More importantly, only formulation with adjuvant A2 provided some protection as evidenced by better ADG and reduced lesions. This formulation is promising and should be studied further.

Keywords

Swine, Vaccine, Immunity

An avidity ELISA for bovine antibodies against *Salmonella* spp.

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Abstract

During the course of an immune response, B lymphocytes that produce antibodies with higher affinity are selectively expanded, resulting in production of antibodies with increasing avidity. This study aimed to develop an avidity test for antibodies that can distinguish between acute salmonella infections and salmonella carriers in cattle.

Control of salmonellosis in Dutch dairy herds focuses on infections caused by *Salmonella enterica* subsp. *enterica* serogroups B and D. Successful control is strongly linked to culling of both active and latent salmonella carriers from persistently infected herds. To identify carriers, biennial serial testing by antibody-ELISA followed by faecal culture of antibody-positive cattle is recommended. Cattle that are both antibody and culture positive are presumed to be active carriers, whereas cattle that remain antibody positive at subsequent tests without a positive faecal culture are suspected of latent carriership.

To improve the timely identification of carriers and reduce testing costs, the antibody ELISA was adapted to an antibody avidity ELISA, that may be used to

1. Identify potential active carriers among antibody-positive cattle, to decrease the number of faecal cultures required in herd tests, and
2. Select potential latent carriers in a single test, to enable earlier removal from the herd.

Our results show that both active and latent carriers exhibit significantly higher *Salmonella* antibody avidity compared to cattle with acute *Salmonella* infections. This discovery could aid in the preselection of potential active carriers for faecal culture and in the prompt removal of both active and latent carriers from the herd.

Keywords

Salmonella, antibody avidity, ELISA

Exploring Students' Perception of Vaccination in the Post-COVID Era

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Abstract

The COVID-19 pandemic has raised questions about mandatory and voluntary vaccinations, which have positive and negative social implications. The study aimed to investigate students' perception of vaccination in a post-COVID era, as well as whether owning a pet and undergraduate background can contribute to vaccination awareness. The questionnaire included demographic data and Likert scale to gauge students' opinions, feelings, and attitudes towards voluntary vaccinations. A survey was completed by 442 students at the University of Belgrade. Among them, 55% had been vaccinated against COVID-19 and 51% would recommend the vaccine to others. The Pfizer vaccine was predominantly used (64%), compared to the other three available manufacturers, which were less utilized (36%). Among students who had a Grammar School bachelorette, 64% were vaccinated, while among those who had a vocational high school bachelorette, 42% were vaccinated ($p < 0.001$). The willingness towards vaccination was mostly influenced by personal understanding (61%), followed by recommendations from a medical doctor (14%), and family members (13%). Media had a low influence (4%) ($p < 0.001$). Ownership of a pet and perception of necessity to vaccinate pets did not influence their decision about vaccination. 30% of students had received non-obligatory vaccines against infectious diseases, and 80% considered vaccination against sexually transmitted diseases to be necessary. It could be concluded that in the post-COVID era, broad education given by Grammar School, as well as personal understanding, but not the pet ownership, influenced favourable opinions and attitudes towards voluntary vaccinations. Furthermore, students possess a positive perception of voluntary vaccination for health protection.

Keywords

questionnaire; undergraduate background; pets

Canine *in vitro*-generated tumor-conditioned macrophages display an M2-skewed phenotype

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Abstract

Tumor-associated macrophages (TAMs) are heterogeneous and abundantly present in the tumor stroma. They derive from tissue-resident macrophages and monocytes and play a central role in cancer. In murine models, TAMs promote invasion, intravasation, circulatory survival, and extravasation of tumor cells. TAMs promote tumor progression and angiogenesis, remodel the tumor microenvironment, and modulate the adaptive immune system. Dogs have proven to be reliable cancer models, and we hypothesized that canine tumor-conditioned monocyte-derived macrophages (TCMΦ) are similar to anti-inflammatory M2 (M-CSF+IL4) macrophages and have a tumor-promoting phenotype, as in humans. We collected blood from healthy dogs, isolated monocytes, and differentiated them *in vitro* using tumor-conditioned media from three canine cancer cell lines. Three TCMΦ populations were generated and compared with *in vitro*-generated M1 and M2 macrophages. We assessed them using multicolor flow cytometry and RNA sequencing. All TCMΦ showed an increased expression of two M2 markers (CD209 and FcεRI), while 2 out of 3 TCMΦ populations had increased expression of two additional M2 markers (CD206 and CD11d). We also identified CD209, CD11d, and LYVE-1 as new phenotypical M2 markers in canine monocyte-derived macrophages. Transcriptomically, several M2-associated surface markers, cytokines, and chemokines were upregulated in TCMΦ, while M1-associated genes were downregulated. In conclusion, we show that canine TCMΦ share many similarities with human TCMΦ, highlighting the translational value of dogs as cancer models.

Keywords

Cancer, tumor-associated macrophages, canine

Conventional and regulatory bovine neutrophil respond differently to *Mycobacterium bovis* clinical strains

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Abstract

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis* (*M. bovis*), is a chronic disease in cattle and a neglected cause of zoonotic human tuberculosis. In developed countries bTB remains a major economic burden. In humans, neutrophils are involved in innate immune resistance to *M. tuberculosis*. Evidence of resistance to *M. bovis* infection was also reported in contact cows, but mechanisms are still unknown. Neutrophils are overlooked in bTB. We recently identified a new neutrophil subset in cattle and mouse that shares the morphology of conventional inflammatory neutrophils, but expresses major histocompatibility complex class II (MCH-II) molecules. Only this later subset is able to suppress T lymphocyte proliferation hence is regulatory. In this study, our objective was to decipher the response of conventional versus regulatory bovine neutrophils to a panel of field *M. bovis* strains circulating in France. We purified bovine blood neutrophil subsets by flow cytometry and infected them *in vitro* by four strains. After two hours, we analysed phagocytosis, killing and reactive oxygen species production. We also deciphered the transcriptomic signature of both subsets. Our results show differences between the two subsets on the different parameters measured as well as different responses depending on the *M. bovis* strain. Therefore, unravelling the role of bovine neutrophil subsets in response to *M. bovis* clinical strains will help gaining better knowledge of bTB physiopathology and may pave the way for the discovery of new biomarkers to follow the clinical status of cattle which is urgently needed to better manage this costly disease.

Keywords

tuberculosis, *Mycobacterium bovis*, neutrophils

Evaluation of *Saccharomyces cerevisiae* as a platform for vaccination against mastitis.

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Abstract

Bacterial intramammary infections (mastitis) represent the most frequent disease of dairy cows and the primary cause of antibiotics use in dairy farming. Vaccines against mastitis are disponible, but their efficiency remains controversial. Thus, the development of new vaccines is necessary. The capacity of yeasts to express heterologous proteins and the immunogenic nature of their cell wall make them an ideal antigen delivery system. We evaluated the potential of *Saccharomyces cerevisiae* as a platform for novel vaccines against bovine mastitis.

First, the ability of *S. cerevisiae* strain EBY100 (ATCC) to stimulate bovine immune system was evaluated *in vitro*, showing that EBY100 induces the production of inflammation and T-cell response markers in blood cells.

The innocuity of EBY100 to mammary epithelial cells (MECs) was observed in *in vitro* and *ex vivo* models, suggesting that a yeast-based vaccine can be administered via the intramammary route and is unlikely to compromise milk production.

Then, EBY100 expressing OVA by yeast surface display was used as a model vaccine to immunise six dairy cows by an intramuscular injection followed by an intramammary booster. None of vaccinated animals showed systemic or local reactogenicity events. The *in vitro* stimulation of their PBMCs after vaccination showed significant IFN γ and IL-17 responses against EBY100 but not against OVA.

Our results point out that *S. cerevisiae* is safe for intramuscular and intramammary immunisation. Nevertheless, futures vaccination strategies exploring *S. cerevisiae* should take into consideration the immunodominance of its antigens.

Keywords

Mastitis, Vaccine, Yeast

Shaping host-pathogen immune responses with *M. bovis* BCG - from extracellular vesicles to trained immunity

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Abstract

Bovine tuberculosis (bTB) caused by *Mycobacterium bovis* continues to have significant financial and social impact on cattle farm businesses worldwide. Highlighted within the Godfray review (2018) of the UK Government's 25-year bovine TB strategy there is a requirement to better understand the principles of bTB pathogenesis and *M. bovis* BCG immunity. Current research has identified that extracellular vesicles (EV) may have important immunological properties in shaping host-pathogen interactions. Limited research has been undertaken to investigate the role of EVs produced by different strains of *M. bovis* BCG and their influence on host derived cells. We characterised EVs derived from *M. bovis* BCG strains; Tokyo and Danish isolated by size exclusion chromatography using transmission electron microscopy and proteomics analysis of surface proteins. EVs were found to range between 20-40nm in size and surface protein preparations contained key antigenic or immunomodulatory proteins including MPB83, PPE family members, and heat-shock proteins. Following exposure to *M. bovis* BCG Tokyo EVs were readily phagocytosed by alveolar macrophages within 24 hours and resulted in pro-inflammatory cytokine production. We aim to study the role of EVs in the development of trained immunity. Innate immune memory is a largely understudied concept in cattle, particularly the molecular and metabolic control. Using *M. bovis* BCG to train CD14⁺ monocytes we observed an increase oxygen consumption rates (indicative of glycolysis), TNF α and MCP-1 compared to naïve controls upon re-stimulation with LPS, Pam₃CSK₄ and *M. bovis* BCG. Taken together, we present functional data that enhances our understanding of *M. bovis* BCG immunity.

Keywords

Mycobacteria, Monocytes, BCG

Cellular responses in British domestic pigs that survive infection with the moderately virulent African swine fever virus strain Estonia2014

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Abstract

Virulent genotype II African swine fever virus (ASFV) is the primary cause of the ongoing ASF panzootic in suids. Urgent development of safe and effective vaccines is imperative, yet understanding of protective immune responses remains limited. Here, we examined cellular responses in convalescent animals following challenge with the moderately virulent genotype II Estonia 2014 isolate.

Twelve pigs, including six inbred Babraham and six outbred domestic pigs, were oronasally inoculated with Estonia 2014. All animals exhibited clinical signs, with one Babraham and three outbred pigs reaching humane endpoints between nine to thirteen days post inoculation (dpi). Additionally, six outbred pigs were mock inoculated as controls. Peripheral blood mononuclear cells were isolated from surviving animals at 14 and 27 dpi and stimulated overnight with antigen to characterize ASFV-specific responses. Using spectral flow cytometry, we phenotyped cell subsets and measured intracellular cytokines.

At 14 dpi, convalescent animals produced T_H1 cytokines, tumor necrosis factor and interferon- γ , in CD4⁺CD8 α β ⁺ cytotoxic T-cells (CTLs) and activated CD4⁺CD8 α β ⁺ T-helper cells. An increase in polyfunctional cytolytic CTLs, expressing the cytolytic marker, CD107a, and producing T_H1 cytokines, was observed at both time points. Interestingly, ASFV-specific responses were more pronounced in Babraham pigs compared to outbred animals, and these responses were more robust at 14 dpi compared to 27 dpi in both breeds.

This study highlights that ASFV recall responses in CTLs and activated CD4⁺CD8 α β ⁺ T-helper cells are key features in Estonia2014 convalescent animals. Further dissection of antigen-specific responses in these samples will facilitate antigen discovery for future ASFV vaccines.

Keywords

African swine fever virus, T-cell response, inbred pigs

Bronchoalveolar T helper cell analysis to characterize equine asthma endotypes

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Abstract

Introduction Equine asthma (EA) is a common chronic obstructive pulmonary disease of adult horses. Affected horses display hyperreactivity to hay dust resulting in bronchospasm, mucus hypersecretion, and lower airway inflammation. EA is categorized into mild-moderate (MEA) and severe (SEA) phenotypes according to clinical signs and bronchoalveolar lavage (BAL) cytology with consistent neutrophilic cytology in SEA. Specification of EA endotypes based on underlying pathogenetic mechanisms has not been established, but type-2 based allergy has been assumed.

Methods Horses were phenotyped by clinical examination, bronchoscopy, and BAL cytology and retrospectively grouped as healthy (HE), MEA, or SEA in three studies (n=5–10/group each). BAL cells and peripheral blood mononuclear cells (PBMC) were analyzed *ex vivo* and after *in vitro* stimulation (PMA, ionomycin). T cells were characterized by flow cytometry (CD4, CD8, intracellular cytokines) comparing the groups and samples.

Results BAL lymphocytes were dominated by CD8⁺ T cells, and CD4⁺CD8⁺ cells, which were not B cells. In contrast, PBMC lymphocytes were dominated by CD4⁺ T helper (Th) cells. BAL CD4⁺IL-17A⁺ Th17 cells were increased in SEA compared to HE. In PBMC, several CD4⁺ Th subsets were increased in SEA and MEA, and expressed CD154, IL-4, IL-10, IFN- γ , and/or TNF- α .

Discussion Increased BAL Th17 cells point to a local type-3 bias in SEA and a non-type-2 endotype. Blood lymphocytes indicate systemic activation in MEA and SEA, but not a clear polarization. Accordingly, BAL T cell analysis appears promising to evaluate asthma endotypes, but this remains challenging in MEA. Endotype definition could inform individualized treatment in the future.

Keywords

Th17, asthma, horse

The loss-of-function of SOCS2 increases the inflammatory response to *Staphylococcus aureus* infection

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Abstract

Introduction/purpose

Mastitis, an inflammation of the mammary gland essentially caused by bacterial infections, is the main disease of dairy ruminants. Whereas the involvement of SOCS2 in anti-infective immune mechanisms has been largely overlooked, we recently identified a loss-of-function point mutation in the suppressor of cytokine signaling 2 (SOCS2) in dairy ewes with a higher predisposition to mastitis.

Methods

We used experimental infections in ewes and in a genetically edited mouse model that expresses the R96C mutation to decipher the role of SOCS2 in the immunity to *Staphylococcus*, a major pathogen of mastitis.

Results

Epidemiologic data and experimental infections in lactating ewes confirmed the increased inflammatory response upon *Staphylococcus* infection. Stimulation of bone marrow-derived macrophages (BMMs) with various TLR-2 ligands in cytokine-modulating environments showed that the absence of functional SOCS2 to be associated with significantly higher levels of the pro-inflammatory cytokines IL-6 and TNF- α . Interestingly, these results are observed only when SOCS2 overexpression is promoted by the presence of GM-CSF. When SOCS2 is inactive, *S. aureus* infection provoked an increase in neutrophil and F4/80^{int} Ly6C⁺ inflammatory macrophage recruitment, as well as a significant increase in IFN- γ and IL-10 concentrations in the murine peritoneal cavity. The SOCS2 protein regulates STAT-5 phosphorylation in a time-dependent manner and drops the inflammatory response and cytokine secretion in favor of better healing during an infection caused by *S. aureus*.

Conclusion

Our results provide evidence that the SOCS2 protein controls inflammatory cytokine production and reduce cell infiltration at the early stage of infection.

Keywords

SOCS2, Mastitis, Inflammatory response

A vaccine for treatment or prevention of verotoxin-producing *Escherichia coli* (VTEC) infection.

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Abstract

Verotoxin-producing E. coli (VTEC) are a group of zoonotic foodborne pathogenic *E. coli* strains that are associated with causing bloody diarrhoea. VTEC infections can result in Haemolytic Uremic Syndrome (HUS) which is the leading cause of kidney failure among young children under the age of 5. Currently, the VTEC notification rate in Ireland is amongst the highest in Europe with 19.0 cases per 100,000. While antibiotic treatment is contradicted due to a large number of patients experiencing severe symptoms and complications, there is an unmet need for a vaccine to reduce mortality and reduce the greatest risk of kidney failure in children. As VTEC colonises gastrointestinal epithelial cells, bacterial adhesin proteins involved in host-cell attachment represent promising vaccine candidates as previously shown to provide protective effects in other infections. We previously used a proteomic approach developed in the lab to identify 14 proteins unique to two VTEC strains as potential adhesins to two gastrointestinal cell lines HT29 or Caco-2. To date, two of these have been tested in mice, one of which, GlnH, reduced bacterial colonisation in the colon and caecum by 1.25-log.

Three additional identified adhesins, antigen A, antigen P, and antigen G were selected for investigation as potential antigens. To date, these antigens were found to be highly prevalent among the bovine and sheep isolates and have been successfully cloned, expressed, and purified for further evaluation in an immunised and oral challenge murine model.

Keywords

verocytotoxigenic *E. coli*, VTEC, vaccine, bacterial adhesin, antigens

Live attenuated and E2-based subunit vaccines against classical swine fever induce different dendritic cell and T cell responses, confirming effective protection by different mechanisms

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Abstract

Classical swine fever virus (CSFV) causes a contagious febrile disease in pigs that is often fatal. Live attenuated C-Strain vaccine induces protection within 5 days. First generation subunit vaccines using CSFV-E2 protein are deemed to have lower efficacy. The subunit vaccine Porvac, comprising poCD154 conjugated to CSFV-E2, has been proposed to offer effective protection similar to C-strain. Here, Porvac and CSFV-E2 alone adjuvanted with ISA50v2 were compared to C-strain pre- and post-challenge with pathogenic CSFV.

All vaccines protected from clinical disease with CSFV. Surprisingly, the dendritic cell related immune responses were very succinctly different and not aligned with textbook expectations: the subunit vaccines induced a predominant cDC1 reaction while the most prominent dendritic cell population responding to C-strain vaccination was cDC2.

While no significant amounts of CSFV-specific T cells could be detected in the lymph nodes of C-strain group pre- or post-challenge, significant T cell responses were observed 7 and 14 days after challenge in the lymph nodes in both Porvac and the E2 groups. However, only in C-strain vaccinated pigs activated CD8⁺ CTL with cytotoxic activity (CD107⁺) were found in the tonsils and in the peripheral blood 4 days post-challenge. Later CD8⁺ CSFV-specific T cells were also detectable in the blood of both subunit vaccine groups.

This study demonstrates the efficacy of modern adjuvants to generate anti-viral immune responses. The study confirmed that subunit vaccines and the live attenuated C-strain use different mechanisms to induce efficient immune responses. Intriguingly, neither the subunit vaccines nor C-strain were operating as expected.

Keywords

Vaccines, Dendritic cells, Adjuvant

Search for biomarkers replacing the rosette test in an immunosuppressed guinea pig model

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Abstract

The animal model of immunosuppressed guinea pigs with azathioprine is standardly used to determine the effectiveness of immunomodulating preparations. The disadvantage of the current model is that the main monitored parameter is the result of the E-rosette test, which is time and technically demanding historical method. The aim of this study was to characterize immunological, hematological and biochemical parameters in order to find parameters that might be suitable as biomarkers of induced immunosuppression. Given that rosette formation has historically been associated with the detection of T lymphocytes, T lymphocyte detection by flow cytometry seemed to be a promising method. The ratio of B and T lymphocytes and the subpopulations of T lymphocytes differentiated based on the expression of CD4 and CD8 markers were monitored in the study. The results obtained by the rosette assay showed a clear reduction in the percentage of rosette-forming leukocytes in the group of guinea pigs with induced immunosuppression. Unfortunately, the percentage of T lymphocytes did not correlate with the rosette test results. Similarly, all hematological and biochemical parameters measured were comparable in the immunosuppressed and control groups. In conclusion, a suitable biomarker could not be found among the parameters studied. Even the use of flow cytometry to detect the percentage of T lymphocytes did not show an association with induced immunosuppression. Thus, the rosette test remains the only suitable methodology for monitoring immunosuppression in guinea pigs. This study was supported by the Ministry of Agriculture (RO0523).

Keywords

guinea pig, immunosuppression, flow cytometry

USE OF FLOW CYTOMETRY IN DIAGNOSIS OF LYMPHOPROLIFERATIVE DISEASES IN GUINEA PIGS

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Abstract

Flow cytometry (FC) is an important tool for the objective diagnosis and further characterization of lymphomas in dogs and cats. Characteristics such as the expression of cell surface markers or cell size can provide important prognostic information. In guinea pigs, lymphoproliferative disorders have mostly been described as single case reports. However, a recent retrospective study showed that lymphomas in guinea pigs were found in 10.46% (16 cases) of a total of 153 tumours diagnosed. Leukocyte immunophenotyping using flow cytometry has been published for guinea pigs yet but was largely limited by the lack of available reagents. The aim of this study was to introduce into clinical practice an optimized compatible multicolour multiparameter FC panel for routine immunophenotyping of peripheral blood and lymph node biopsies in guinea pigs with suspected lymphoproliferative disease. The panel of commercially available monoclonal antibodies enables the determination of CD4+ and CD8+ T-cells as well as T- and B-cell populations. As an example, its usefulness is presented in clinical cases of guinea pigs with cervical masses. Flow cytometric analysis revealed a large B-cell lymphoma and, in another case, a predominance of small, non-leukocytic cells together with B- and T-lymphocytes in tested sample. The histopathological examination confirmed the diagnosis of a lymphoma and thyroid gland adenocarcinoma with an inflammatory lymphocyte infiltrate, respectively. The application of this powerful method makes it possible to increase the accuracy of the diagnosis of lymphoproliferative diseases in domestic and experimental guinea pigs. This study was supported by the Ministry of Agriculture (RO0523).

Keywords

lymphoma, immunophenotyping, guinea pig

Impact of swine influenza virus on porcine reproductive and respiratory syndrome virus infection dynamics in alveolar macrophages

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Abstract

Respiratory infections represent a major challenge for the swine industry, with swine influenza A virus (swIAV) and porcine reproductive and respiratory syndrome virus (PRRSV) being major contributors. Epidemiological studies have confirmed co-circulations of these viruses in pig herds, making swIAV-PRRSV co-infections to be expected.

Here, we investigated the impact of swIAV on primary alveolar macrophages (AMs), and its effects on PRRSV infection, either when cultivated in monoculture or in co-culture with respiratory epithelial cells (NPTr cell line). In co-culture system, AMs are primarily targeted by PRRSV, while NPTr by swIAV. AMs were obtained either from conventional or specific pathogen-free (SPF) pigs.

swIAV replication was abortive in AMs, inducing cell death at high multiplicity of infections. In AMs from three out of four conventional animals, swIAV showed no impact on PRRSV replication. However, inhibition of PRRSV multiplication was observed in AMs from one animal, accompanied by an early increase in interferon (IFN)-I production and expression of IFN-stimulated genes. In AMs from six SPF pigs, swIAV inhibited PRRSV replication in all batches, with an early induction of antiviral genes. Co-culture experiments involving NPTr cells and AMs from either SPF or conventional pigs all showed swIAV-induced inhibition of PRRSV replication together with early induction of antiviral genes.

Overall, this study revealed a plausible role of animal sanitary status in the interaction of swIAV on PRRSV propagation in AMs, justifying further investigation on viral interactions and role of the respiratory innate immune system memory.

Keywords

Alveolar macrophages, swine influenza A, porcine reproductive and respiratory syndrome virus

Microbiome-immune interactions in the bovine udder analyzed through a full production cycle

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Abstract

The correct detection of subclinical mastitis remains a major hurdle in dairy production. Cultivation of bacteria may miss large parts of the udder microbiome, and somatic cell counts (SCC) only crudely testify of the host response. In this study, we followed the microbiome and immunological components in the milk at the quarter level in 24 clinically healthy cows over one year, including before and after a dry period. We used amplicon and shotgun metagenomics sequencing as well as measurements of the host immune response by flow cytometric phenotyping and detection of cytokines and chemokines in the milk. Integrated analyses reveal patterns of host responses to distinct taxa in the microbiome, which may include opportunists, pathogens and co-infections, at different times in the production cycle. Furthermore, the physio-chemical properties of the milk were recorded to discover situations whereby the milk quality may be compromised. Knowledge of microbe-host interactions in the udder may reveal pathogenic species that are not detectable in culture and may cause immune responses. Finally, knowledge of drivers of different cellular components in the SCC will inform the interpretation of SCC numbers and highlights the utility of differential somatic cell counting to screen for subclinical mastitis more accurately.

Keywords

bovine; microbiota; mastitis;

Exploring age related effects on the immune response of resistant Canaria Hair Breed lambs to *Teladorsagia circumcincta*

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Abstract

Effective immune responses against gastrointestinal nematodes (GIN) in young lambs of many commercial breeds are usually acquired at the end of the first grazing season. However, there are some breeds that display ‘natural’ resistance against GIN and demonstrate protective mechanisms at a younger age. In the Canary Islands (Spain), the indigenous Canaria Hair Breed (CHB) proved to be resistant to repeated infections of *Teladorsagia circumcincta* at 6 months-old, by reducing worm length and number of eggs in female worm uteri and showing lower FEC than another local breed (Canaria Sheep (CS)). Such resistance was not perceived at 3 months of age, with no significant differences in parasitological parameters measured in CHB compared to CS lambs in a study performed under similar conditions. In this work, we compare the data obtained at both ages, exploring differences in parasitology and immune responses in CHB lambs subjected to repeated infections with *T. circumcincta* larvae at 6 (Trial 1) and 3 months-old (Trial 2). Faecal samples were taken during the experiment, while abomasal worm burdens and tissue samples were obtained post-mortem. Six-month-old lambs showed significantly higher CD4, CD8, gamma-delta, CD45RA, MHC-II and Gal-14 cell populations than 3 month-old lambs. This could suggest that 3 month-old lambs were not able to mount an effective response due to insufficient immune coordinator cells. Understanding the mechanisms behind the protective response of resistant animals at a young age could be a key factor for future vaccine development and genetic selection.

Keywords

GIN, resistant sheep breed, cellular response

Inflammation associated biomarkers in serum as prognostic marker for disease progression in calves infected with bovine Respiratory Syncytial Virus

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Abstract

Bovine Respiratory Syncytial Virus (bRSV) is a respiratory pathogen leading to acute respiratory disease in young calves. The immune system plays a role in the bRSV-specific pathogenesis. bRSV triggers a mixed cytokine response with increased levels of proinflammatory cytokines (e.g. IL-6, TNF α , IFN γ), and enhanced levels of IL-4. Imbalanced Th1/Th2 responses are linked to disease severity but the precise role of the immune system in bRSV-specific pathogenesis and the variability of the respiratory disease is still unknown. The aim of this study is to define a profile of inflammation associated biomarkers in serum that is predictive for disease progression in bRSV-infected calves.

For this purpose, cytokine and chemokine levels were determined in serum samples from experimentally infected calves using the MILLIPLEX[®] bovine cytokine/chemokine multiplex assay. C-reactive protein and haptoglobin levels were assessed by ELISA. K-means (k=5) clustering with dynamic time warping as distance metric was applied to identify patterns unsupervised.

bRSV infection resulted in increased levels of IFN γ , IL-1 α , IL-1 β , IL-4, IL-6, IL-8, TNF α , MIP-1 α , CXCL10, macrophage chemo-attractant protein (MCP)1, VEGF-A and haptoglobin between day 0 and day 9 post infection. The clustering indicates that a transient peak in serum levels of IFN γ and MCP1 is associated with recovery, while prolonged levels of IL-1 α are related to progressive disease.

In conclusion, we identified a specific profile of inflammation associated markers indicative of progressive disease in bRSV infected calves. Analysis of more animals is currently in progress to validate these preliminary results.

Keywords

bRSV, cytokines, clustering

Leukogram patterns and monocyte subtypes in blood collected from laying hens during erysipelas outbreaks

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Abstract

Outbreaks of erysipelas, i.e. systemic *Erysipelothrix rhusiopathiae* (ER) infections, is a re-emerging problem in layers with outdoor access. This study aimed to monitor blood leukocyte profiles of hens undergoing natural ER infections.

Blood was collected from 77 hens in four flocks affected by acute outbreaks of erysipelas. Presence of ER in blood was quantified by culture and absolute differential leukocyte counts including lymphocyte subtypes were determined by flow cytometry. Principal component analysis (PCA) was performed on leukocyte data.

Results showed that hens with “high” bacteraemia ($>10^6$ cfu/ml, n=6) predominantly had low numbers of monocytes, lymphocytes of all subtypes and thrombocytes, high expression of CD45 on heterophils and monocytes with a MRC1L-B^{high}MHCII^{low} profile. Hens with “medium” ($>10^4$ cfu/ml, n=4) and “low” ($<10^4$ cfu/ml, n=6) bacteraemia had high numbers of heterophils and monocytes. Hens negative for ER (n=61) predominantly had monocytes with a MRC1L-B^{low}MHCII^{high} profile. A mixed group of medium, low and negative hens had monocytes with a MRC1L-B^{low}MHCII^{low} profile. The PCA clustered high and medium hens separately from negative hens while low hens were found in both clusters. Monocyte expression of MRC1L-B, heterophil expression of CD45, heterophil and monocyte numbers were identified as important parameters to distinguish ER positive hens and monocyte MHCII and CD45 expression were important in distinguishing ER negative hens.

Hence, we identified distinct leukogram patterns in hens with different levels of ER bacteraemia. For example, monocyte subtypes were notably altered by infection and interestingly heterophilia was not prominent in hens with high bacteraemia.

Keywords

chicken, erysipelas, leukocytes

Vaccination against paratuberculosis triggers trained immunity mechanisms that may induce protection against other pathogens

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Abstract

Mycobacterium avium subsp. *paratuberculosis* (Map) is the aetiological agent causing paratuberculosis, a severe chronic granulomatous enteritis that affects ruminants worldwide generating large economic losses. Vaccination although limited in many countries, has been posed as an effective cost-benefit measure against PTB. Furthermore, an inactivated Map vaccine (IMV) has shown heterologous protection and features suggesting trained immunity in epidemiological studies. The aim of this work is to demonstrate by functional assays and epigenetic analysis that this vaccine triggers mechanisms of trained immunity in calves. Therefore, 15 day old vaccinated (n=5) and non-vaccinated (n=5) calves were included in the study to evaluate and compare specific and non-specific innate immune responses. Vaccinated calves showed a significant increase in neutrophil-mediated phagocytic activity and neutrophil-mediated reactive oxygen species (ROS) production measured by flow cytometry one, two and three months after vaccination, whereas ROS mediated by monocytes, was significantly increased only after 3 months. Neutrophil extracellular trap release observed by in vivo imaging analysis, showed an increase in vaccinated calves after 2 hours of stimulation against Map, *Mycobacterium bovis*-BCG, *Escherichia coli* and *Staphylococcus aureus*, which was maintained after 4 hours of stimulation with BCG and *S. aureus*. An increase in lactate over time, with higher values in the vaccinated group, especially 3 months post-vaccination confirmed a metabolic shift. These results together suggest that IMV does induce trained immunity, with innate cell activation maintained for at least three months after vaccination, pending the ChIPseq results on H3k4me3 accumulation in gene promoters related to trained immunity for complete confirmation.

Keywords

trained immunity, neutrophils, mycobacteria

Domain orientation in CLR-Fc fusion proteins affects ligand binding strength

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Abstract

Myeloid C-type lectin receptors (CLRs) are a family of pattern recognition receptors recognizing glycans derived from bacteria, viruses, fungi, parasites, thus initiating innate responses as well as shaping adaptive immunity. CLR-Fc fusion proteins, consisting of the extracellular domain of the CLR (comprising the carbohydrate recognition domain [CRD]) fused to the Fc domain of IgG, are important tools employed in studies aimed at identifying novel CLR/pathogen interactions.

This study aimed at comparing the N- vs. C-terminal fusion of the Fc domain and its influence on the binding activity of CLRs. Working with the vectors pFUSE-hIgG1-Fc2 (for C-terminal fusion) and pFUSEN-hG1Fc (for N-terminal fusion), Fc-fusion proteins for the CLRs human DC-SIGN/CLEC4L, mouse Mincle/CLEC4E and mouse Dectin-1/CLEC7A were prepared. The fusion proteins were compared using ELISA-based binding assays against the S-layer glycoprotein from *L. brevis* and heat-killed *Candida albicans* (HKCA). In the case of the S-layer glycoprotein, the N-terminal fusion of the Fc domain to the three tested CLRs yielded a significantly stronger binding. In the case of HKCA, the N-terminal fusion also produced a significantly stronger binding, but only for Mincle and Dectin-1.

In conclusion, our results suggest that fusion to the N-terminal flexible neck region of a CLR may be preferred in some instances than fusing to its C-terminal CRD, especially for the detection of weaker interactions. They also underline that careful considerations are warranted when designing fusion proteins, such as the orientation of the fused domains, and that those aspects should be evaluated for applications demanding stronger binding activities.

Keywords

C-type lectin receptors, Fc-fusion proteins

Dissecting the neutralising antibody response to porcine reproductive and respiratory syndrome virus to identify novel vaccine targets

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Abstract

Porcine reproductive and respiratory syndrome (PRRS) continues to cause economic losses to the global swine industry. The causative PRRS viruses (PRRSV) exist as two rapidly evolving species causing recurrent outbreaks which cannot be adequately controlled by existing live attenuated vaccines. Characterising the targets of broadly neutralising antibodies is of great interest and should guide next-generation vaccine design.

Recombinant soluble forms of PRRSV-1 minor envelope glycoproteins (GP2-4) were expressed and assessed for antigenicity using serum isolated from pigs sequentially infected with diverse PRRSV strains. Since GP3 was well recognised, GP3-based tetramers were constructed to sort single antigen-specific B cells, however, the resulting recombinant anti-GP3 monoclonal antibodies (mAb) failed to neutralise PRRSV *in vitro*. A parallel evaluation of GP3 immunogenicity, including the glycoprotein delivered on adjuvanted liposomes (CoPoP); in an oil adjuvant, or delivered via a self-amplifying RNA vector, failed to induce a measurable neutralising antibody response, despite high antigen-specific antibody titres.

As an alternative strategy, commercial sows repeatedly vaccinated against PRRSV were identified as possessing antibodies capable of broadly neutralising PRRSV-1 and -2. Ongoing experiments are being performed to assess the effect of depletion of PRRSV glycoprotein specific antibodies from the serum on virus neutralisation *in vitro* to prioritise antigens for single cell sorting and mAb isolation. Purified PRRSV-1 and -2 virions have been fluorescently labelled to offer a second complimentary unbiased approach. It is hoped these approaches will enable the isolation of neutralising and broadly neutralising mAb, which could be used to define highly conserved epitopes on PRRSV glycoproteins

Keywords

Porcine reproductive and respiratory syndrome virus, glycoprotein, neutralising antibody, vaccine

***Aspergillus fumigatus* in severe equine asthma – Antigen identification and serology to elucidate etiology?**

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Abstract

Introduction: Severe equine asthma (SEA) is a prevalent airway disease of horses. *Aspergillus fumigatus* (*A. fumigatus*) is common in hay and has been described as a provoking agent of SEA, but single relevant antigens have not been identified comprehensively.

Methods: First, we explored *A. fumigatus* as a source of candidate antigens in an immunoproteomics approach. Proteins of *A. fumigatus* were extracted, separated by two-dimensional gel electrophoresis, and immunoreactivity was tested on immunoblots using serum from healthy horses (HE) and horses with SEA (n= 5 each). Spots that yielded differential serum antibody binding (all Ig 'Pan-Ig', IgG4/7, IgG3/5) between HE and SEA were picked and proteins contained were identified by LC-MS. These were tested as recombinant (*r*) antigens on immunoblots for serum Ig binding. Second, confirmed antigens were compared to *A. fumigatus r* allergens using ELISA with different sera from HE (n = 18) and SEA (n = 23) and binding of eight Ig isotypes was assessed.

Results: Four new antigens were identified by immunoproteomics. Immunoblots usually showed higher serum IgG3/5, but not increased Pan-Ig binding in SEA compared to HE. In contrast, the cohort tested by ELISA yielded higher serum IgG1, but not IgG3/5 or IgE, binding in SEA. Moreover, all tested *A. fumigatus* antigens and allergens resulted in similar Ig binding patterns using ELISA.

Discussion: *A. fumigatus* showed high immunogenicity but dominance of single antigens remains to be confirmed. Dominating Ig isotypes in SEA need to be verified due to methodical differences but hold potential to aid understanding equine asthma pathogenesis and etiology.

Keywords

antibody isotypes, Western blot, horse

Intradermal electroporation of naked mRNA vaccine elicits an antigen-specific protective immune response in animal models

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Abstract

Recent concerns over the side effects associated with lipid nanoparticles (LNPs) used in mRNA vaccine delivery have highlighted the need for alternative mRNA delivery methods without LNP. Since electroporation is well-established as an effective method for delivering nucleic acids including DNA vaccines, we investigated whether delivering naked mRNA vaccines *via* intradermal electroporation could induce sufficient immune responses. First we optimized intradermal electroporation conditions using luciferase-encoding mRNA. Next, the immunogenicity and efficacy of a naked mRNA vaccine encoding the SARS-CoV-2 spike protein delivered through intradermal electroporation was investigated in mouse and mini-pig models. In mice, the vaccine-induced significant humoral and cellular immune responses, effectively preventing lethal SARS-CoV-2 infection. Similarly, in mini-pigs, vaccination led to a significant increase in specific antibodies against the spike protein. These findings highlight the potential of LNP-free naked mRNA vaccines delivered by electroporation as a safer alternative to conventional methods, offering effective immunization against SARS-CoV-2 while avoiding the side effects associated with LNPs. This approach could pave the way for developing safer and more efficient vaccines for a range of pathogens.

Keywords

Electroporation, intradermal injection, Naked mRNA Vaccine

Increased *Aspergillus fumigatus*-binding IgG1 and IgA in bronchoalveolar lavage fluid in equine asthma – Not simply allergic?

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Abstract

Introduction: Horses exposed to hay dust often develop equine asthma (EA), a common airway disease in horses. *Aspergillus* (*A.*) *fumigatus*, frequently found in moldy hay, is a confirmed EA stimulus; however, it is unclear if the underlying pathogenesis of EA is allergic. We aimed to analyze equine immunoglobulin (Ig) isotype binding to *A. fumigatus* antigens comprehensively, both locally in the lung (bronchoalveolar lavage fluid, BALF) and systemically (serum).

Methods: In an ELISA approach, plates were coated with one of eight recombinant *A. fumigatus* antigens or *A. fumigatus* lysate. After incubation with either BALF or serum from healthy (n=18), mild to moderately asthmatic (MEA, n=20) or severely asthmatic (SEA, n=24) horses, *A. fumigatus*-binding equine isotypes (IgG1, IgG3/5, IgG4/7, IgG6, IgA, IgE) as well as overall binding Ig (Pan-Ig) were detected. Total Ig isotype content of the samples was determined by bead-based assays.

Results: Compared to healthy horses, *A. fumigatus*-binding Pan-Ig, IgG1 (in BALF and serum) and IgA (in BALF) were increased in asthmatic horses. Binding of other isotypes, including IgE, were similar in all groups. Horses with MEA and SEA showed overall similar Ig binding. Total serum IgG4/7 content was increased in MEA, and BALF IgG1 and IgG4/7 contents were elevated in SEA compared to healthy horses.

Discussion: IgG1 and IgA, but not IgE, appear to be relevant isotypes in neutrophilic EA. This questions allergy/type I hypersensitivity as the only mechanism underlying EA. However, equine IgG1 can activate complement and could contribute to type III hypersensitivity.

Keywords

aspergillus, lung, immunoglobulin isotype

Effect of Single and Simultaneous Vaccination of rHVT-F(ND) and rHVT-H5(AI) on Immune Responses and Protection upon Challenge with Avian Orthoavulavirus-1

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Abstract

Newcastle disease (ND), caused by Avian Orthoavulavirus-1 (AOaV-1), has historically caused numerous outbreaks, leading to the implementation of mandatory vaccination programs in many countries. In recent years, avian influenza (AI) has emerged as a significant threat causing widespread outbreaks, prompting discussions about the need for vaccination strategies against AI. Given the existing vaccination programs against ND, one potential approach is to incorporate AI vaccination into the existing ND vaccination program by using vector vaccines that can be administered simultaneously. In this study, we aim to investigate whether combining vector vaccines against both ND and AI affects the protection against ND compared to using only the vector vaccine against ND.

This study included three groups: 10 layers vaccinated with a recombinant herpesvirus (rHVT) vector vaccine against ND (rHVT-F(ND)), 10 layers vaccinated with both rHVT-F(ND) and an rHVT vector vaccine against AI (rHVT-H5(AI)), and 9 mock-vaccinated layers as the control group. All layers were vaccinated as day-old and challenged with a lentogenic AOaV-1 at 14 weeks of age. Tracheal and cloacal swabs as well as environmental samples were collected at 2, 3, 4, 7, 11 and 14 days post inoculation (DPI) and analyzed for detection of AOaV-1 by rRT-PCR. Blood samples were collected at 3, 7 and 14 DPI and tested by ELISA to investigate for seroconversion.

Preliminary results show that both single vaccinated and double vaccinated groups produced high antibody titers and showed low viral shedding and viral loads in the environment compared to the unvaccinated control group.

Keywords

Newcastle disease, Avian influenza, vaccination

Allergen specific immunotherapy for equine insect bite hypersensitivity, a pilot study

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Abstract

In horses, insect bite hypersensitivity (IBH) occurs after midge bites and manifests as a seasonal IgE-mediated skin allergy with swelling, thickening and induration of the skin, alongside intense pruritus and scratching. Current symptomatic treatments offer temporary relief but carry risks of immunosuppression and side effects. In contrast, allergen-specific immunotherapy (AIT) addresses the underlying allergy mechanism, potentially providing lasting relief. However, no established AIT protocol exists for IBH.

This study aimed to develop an effective AIT for IBH. Previously, *Culicoides obsoletus* was identified as the most relevant species for IBH. Ten *C. obsoletus* allergens were produced as purified recombinant proteins. Four of these allergens (Cul o1P, Cul o2, Cul o3 and Cul o5) showed dominant IgE-reactivity in the sera of two IBH-affected Shetland ponies, and were selected for AIT. The ponies received 7 intradermal AIT injections with these four allergens adjuvanted with monophosphoryl lipid A. Post-treatment, both ponies exhibited increased allergen-specific IgG and reduced allergen-specific IgE when compared to pre-AIT levels. Skin reactivity to the recombinant allergens also decreased, but reactivity to *C. obsoletus* whole body extract (WBE) only reduced in one pony and IBH symptoms persisted after natural midge exposure.

In summary, AIT successfully desensitized the IBH-affected ponies towards the recombinant allergens used, but lacked efficacy in reducing skin reactivity to *C. obsoletus* WBE and alleviating symptoms post midge exposure. Currently, we are enhancing AIT by including newly identified *C. obsoletus* allergens that show major IgE-reactivity in the IBH-affected horses.

Keywords

horse, IBH, allergen-specific immunotherapy

In vitro lymph node cultures to monitor adaptive immune responses in pigs

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Abstract

In recent years, there have been growing efforts to develop *in vitro* models for components of the immune system. For example, the development of human tonsil organoids allowed investigations on T and B cell recall responses and germinal centre formation. We adapted this system using cell preparations from porcine lymph nodes, isolated from pigs vaccinated twice with a commercial inactivated swine influenza A virus vaccine. Conventional IFN- γ ELISpot assays with cells isolated from lymph nodes indicated only marginal responses above the medium control. However, in transwell cultures with the same cell preparations and followed for up to 15 days, a substantial expansion of CD4 T cells with an activated phenotype (CD3⁺CD4⁺ICOS⁺Ki-67⁺) and plasma cells (CD79 α /Pax5⁺/IRF4^{high}Blimp-1⁺) was observed following vaccine antigen restimulation, compared to medium controls. Responses peaked at day 10 of *in vitro* cultivation, but these cultures did not support survival or expansion of germinal centre B cells, identified by a CD79 α ⁺Pax5⁺IRF4⁺Blimp-1⁺Bcl-6⁺ phenotype. Hence, recent work focused on supplementing cultures with different combinations of IL-21, BAFF, and soluble CD40L to improve B cell survival and proliferation. High concentrations of all three proteins together led to substantially improved survival of plasma cells but impaired CD4 T cell responses and reduced antigen-specific recall. Omission of IL-21 improved CD4 T cell recovery, yet reduced plasma cells survival. Low IL-21 concentrations led to antigen-specific expansion of both plasma cells and T cells. These results show that lymph node culture systems have a great potential to study vaccine antigen recall reactions in hitherto unexplored detail.

Keywords

Organoid culture system

LPS induces sphingolipids alteration in cow whole blood as observed after calving.

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Abstract

Sphingolipids are key compounds in inflammatory and immunity responses. Whole blood measurements are used to reveal pathologies, but its applicability in demonstrating sphingolipidome alteration in cattle is unknown. Monovette blood samples were taken from 45 cows on two farms (15 and 30 cows respectively), before and after calving. Blood samples were stimulated for 24h with LipoPolySaccharide (LPS) at 3µg/mL, with controls receiving only LPS. Targeted analysis of sphingolipids was conducted by UHPLC-MSMS. Cytokines were measured by a Merck-Millipore 15-plex bovine cytokine assay. Before calving, LPS stimulation significantly increased sphingosine-1-phosphate (d18:1P) release by 29.4%, and decreased sphingosine (d18:1) and deoxysphingosine (m18:1) bases by 3.3 and 10.6% respectively. Calving alone resulted in sphingolipids changes characterized by a significant increase of d18:1P and dihydrosphingomyelins by 43.3% increase in 39.9%, accompanied by an 27.9% decrease in dihydroceramides. The effects of LPS after calving were similar to those observed on d18:1 and m18:1 before calving, but the increase in d18:1P was only by 19.1%. In addition, LPS caused an increase in cytokine levels before and after parturition, but this effect was not correlated with the change in sphingolipid levels. Taken together, these results show that the effects of parturition on the plasma sphingolipidome are similar to those observed when whole blood is stimulated with LPS, and that parturition reduces the capacity to respond to LPS. They suggest that the sphingolipidome assay in whole blood is a good model for revealing the impact of pathologies that directly involve d18:1P, and dihydrosphingolipids.

Keywords

sphingolipids; cattle; calving; LPS; whole blood

A new Montanide™ adjuvanted autogenous vaccine against bovine papillomatosis

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IZSUM "Togo Rosati", Perugia, Italy

Abstract

Bovine papillomatosis is a constantly growing pathology; in Italy it's possible to fight the spread of the pathology through the use of an autogenous vaccine authorized by the Ministry of Health and produced in the Pharmaceutical laboratories of the IZSUM "Togo Rosati" in Perugia. The main genotypes found in the Bovidae family in Italy are BPV-1, 2, 7 and 13. From our experience, BPV-1 appears to be the most frequently identified from lesions and surgically removed pathological material. A new Montanide™ Seppic IMS1313-adjuvanted inactivated viral autogenous vaccine was carried out. The viral matrix was inactivated with formaldehyde 37% (0.04% v/v), trited, homogenized and subjected to several centrifugation (5000 G) and purification steps by 1,2 to 0,22 μ m filter. The adjuvant IMS 1313 was subsequently added. The administration protocol was single "one shot" administration. 240 adult cattle were involved in the experimentation to evaluate the safety and effectiveness of the new vaccine. The "one shot" vaccine was used on a farm largely affected by a widespread form of bovine papillomavirus type 1 (BPV-1). The results were extremely satisfactory in terms of safety (100%) and of effectiveness (85%). The "one shot" protocol showed slightly lower results compared to the classic three inoculations (85% versus 95% remission) but allowed a significant reduction in costs and working times, aspects which condition the possibility of carrying out the vaccination in some types of farming. This Research was carried out with the funding of the Italian Ministry of Health - IZSUM112021RC

Keywords

papillomatosis, autogenous vaccine, cattle

Neonatal piglets can develop a protective immune response after vaccination with a *Streptococcus suis* bacterin but not with subunit-adjuvanted vaccines

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Abstract

Vaccination within the first week of life could be essential to protect weaning pigs against invasive *Streptococcus suis* (*S. suis*) disease. In this period *S. suis* infections cause high morbidity in pigs, reduce animal welfare and contribute substantially to the use of antibiotics. Alternative effective tools are needed to fight this disease. In this study, we investigated if neonatal piglets could develop a protective immune response after vaccination. Five groups of 5-day-old piglets were intramuscularly vaccinated with: 1; a subunit vaccine with conserved *S. suis* type 2 immunogens MRP2 and C05 adjuvanted with CAF[®]01 (n=8) or 2; CDA (n=8), 3; a non-adjuvanted group (only MRP2/C05, n=5), 4; a *S. suis* bacterin adjuvanted with Specol[®] (n=8) and 5; a non-vaccinated control group (n=4). Animals received their booster immunization 4-weeks later. Three weeks after the booster, piglets were intranasally challenged with *S. suis* serotype 2 (S10-10⁹ CFU) and left for clinical observation up to 8 days after challenge. Blood samples and tonsillar swabs were collected throughout the study to assess humoral/cell-mediated immune responses and bacterial burden. The subunit vaccines adjuvanted with CDA or CAF[®]01 elicited a weak immune response, either humoral or cell-mediated, with no protection after *S. suis* challenge. However, nearly all piglets immunized with the Specol[®]-adjuvanted bacterin were protected after the challenge, with an evident humoral response. This highlights that protection against *S. suis* after neonatal vaccination could be achieved and that further research is needed to find the optimal neonatal subunit-vaccine formulation giving a broad protection.

Keywords

Streptococcus suis, neonatal, vaccination

Equine sarcoidosis: a new vaccine for a novel vaccination approach - preliminary data

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IZSUM "Togo Rosati", Perugia, Italy

Abstract

Equine sarcoidosis is really difficult to fight with the tools available to date. Surgical therapy proves to be particularly difficult also due to the frequency of relapses. At the moment the best path to follow would seem to be that of vaccination as prophylaxis or metaphylaxis. For this purpose, it was decided to test a new approach using a heterologous viral autogenous vaccine in all confirmed cases of equine sarcoid caused by bovine papillomavirus type 1 (BPV-1). Several horses were involved in the study but were recruited only the animals with ongoing sarcoidosis, at least one relapse in the last two years, at least one surgical treatment in the last year, absence of signs of improvement after systemic or topic drugs, BPV-1 positive PCR on biopsy tissue and histological examination concordant with the macroscopic lesions. Five animals were finally enrolled in the study and the informed consent of the owners and the attending veterinarian was obtained. Five heterologous autogenous vaccines containing BPV-1 were prepared and administered according to a vaccination protocol which included three vaccine administrations spaced two weeks apart on the other. The preliminary results seem to indicate a good response of the immune system to vaccine stimulation with a slowdown and in some cases an improvement in clinical conditions even if the study (follow up) is currently underway and further time will be needed to carefully evaluate all the final results. This Research is carried out with the funding of the Italian Ministry of Health - IZSUM112021RC

Keywords

equine sarcoidosis, autogenous vaccine, BPV-1

Caninization of rabbit antibody by CDRx platform

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Abstract

Humanization is a common process that converts an antibody discovered from mouse / rabbit immunization for human therapy. There is an increase demand to convert antibodies from mouse, rabbit or human to animal antibodies to treat diseases in animals. With extensive experiences and 99% success rate in humanization, Fusion Antibodies performed caninization using the CDRx platform to explore feasibility of this conversion.

We started with an antibody sequence which was produced via rabbit B-cell cloning. Initially we searched databases for mature canine frameworks that share homology and key residues with the parental rabbit heavy and light chain. These frameworks and closest canine germlines were compared to the parental antibody in order to make a selection of 5 heavy and 6 light acceptors.

When the frameworks were selected, we performed CDR grafts and subsequently made rational back mutations to maintain the canonical loop structure of the CDR's. Our back mutations are model lead with preference given to the homology model of the parental antibody in terms of CDR structure. The resultant variants were screened for liabilities by algorithm and then produced in a matrix to enable the greatest chance of success.

The full matrix and the parental were produced transiently using the ExpiCHO cell line and purified using a 2-step affinity purification method. The lead antibodies were assessed via Bio-Layer Interferometry on an Octet system in order to confirm binding. We confirmed that caninization using CDRx platform is a feasible and robust process and can apply to other species conversion.

Keywords

Caninization, CDR grafting, Antibody species conversion

Evaluation of MONTANIDE™ GR01, a new adjuvant for feed-based vaccines, on the immune response and protective efficacy against streptococcosis in Nile tilapia (*Oreochromis niloticus*)

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Abstract

In this study, the use of MONTANIDE™ GR01, a novel adjuvant for oral vaccination, which incorporates a matrix serving as a protective barrier for the antigen to protect it from the gastric environment, was investigated for Nile tilapia.

The vaccine against *Streptococcus agalactiae* was prepared and combined with MONTANIDE™ GR01 to evaluate the immune response and protective efficacy following oral delivery via in-feed administration, compared to an unadjuvanted vaccine. Two doses of MONTANIDE™ GR01, at 20% (w/w) and 2% (w/w), included in the adjuvanted vaccines administered through fish feed, were comparatively evaluated in the laboratory trial. The ability to enhance the non-specific immune response, thought to be involved in protective responses, as well as specific antibody (IgM) levels was evaluated. Further evaluation of the formulations involved testing their protective efficacy in vaccinated fish against the live pathogen, utilizing both injection and immersion challenge methods. Importantly, to assess its practical effectiveness, the performance of MONTANIDE™ GR01 as an adjuvant in feed-based vaccines was studied in farm-scale trials, using a single inclusion dose (2%) chosen based on laboratory trial results.

The results indicated that a feed-based vaccine containing MONTANIDE™ GR01 as an adjuvant can stimulate innate immune parameters, the expression of immune-related genes, the levels of specific IgM antibodies and disease protection, showing significantly higher efficacy compared to fish administered a feed-based vaccine without adjuvant under laboratory and on-farm conditions. Therefore, MONTANIDE™ GR01 is an extremely beneficial adjuvant used in oral vaccination strategies to protect Nile tilapia from streptococcal disease.

Keywords

Streptococcosis; Feed-based vaccines; Oral vaccination;

Comparative Immune Competence Analysis of Three Local Chicken Breeds

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Abstract

Both genetics and husbandry management are fundamental to animal health. It is often said, without any scientific evidence, traditional livestock breeds are healthier and have a better immune system than high performance lines. However, little is known about the immune competence of local breeds. The aim of this project is to assess the suitability of three phylogenetically divergent local chicken breeds (Altsteirer, Ramelsloher and Bielefelder) for organic farming with its particular animal health challenges due to free-range husbandry. The breeds were selected based on their regional distribution, with one breed representing the north, south and centre of Germany. They were tested mainly by flow cytometry for their general immune competence and the kinetics of the immune system development after hatching, as well as their resilience to viral infections.

Significant differences in the development of the juvenile immune system were found between the breeds. Unexpectedly, all Ramelsloher day-old chicks (DOC) already have peripheral T-cell levels comparable to adult chickens; Bielefelder DOC have no peripheral T-cells, as expected, whereas Altsteirer show an intermediate phenotype.

To assess viral resilience, six-week-old chickens of each breed were infected with an H5N1 avian influenza virus, which serves as a model virus. Again, breed differences in clinical signs, mortality and transmission were observed. The Ramelsloher had the lowest clinical score and the highest survival rate. Chicken-to-chicken transmission was only observed in the Altsteirer breed.

Our results indicate differences in the immune competence of local chicken breeds and provide breeding recommendations for organic farming.

Keywords

local chicken breeds, immunology, avian influenza virus

Development of a Systems Immunology Approach to Explore Factors Influencing Vaccination Response in Belgian Blue Cattle

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Abstract

Response to vaccination varies significantly among individuals and is influenced by various factors, including genetic and environment. In particular, persistent viruses, such as herpesviruses, appear to profoundly impact the immune response of their host. While these factors have been largely studied in humans, nothing is known in domestic animals. Here, we investigated the effect of genetics and of Bovine Herpesvirus 4 (BoHV4) infection on the responses of calves to vaccination. Briefly, 227 Belgian Blue calves, aged 1 to 6 months and housed under uniform conditions at the Ciney cattle selection center, were vaccinated against *Clostridium perfringens* toxins. Antibody levels were measured four weeks post-vaccination using commercial ELISA kits. BoHV-4 infection status was determined through serology and qPCR. Genotyping was conducted for all calves, and a genome-wide association study (GWAS) was performed to identify genetic factors associated with variation in the responses to vaccination. The findings unveiled significant variations, particularly in response to *Clostridium perfringens* ϵ and β toxins vaccination, with BoHV4-infected calves exhibiting a more robust response. Moreover, the GWAS pinpointed a specific region on chromosome 23 linked to the response to *Clostridium perfringens* ϵ -toxin. This region encompassed genes related to the TNF family and MHC classes I and III. Further research is essential to establish causal links between these identified factors and response to vaccination. Nevertheless, this study paves the way for a detailed understanding of immune response variability in Belgian Blue breed in particular and more generally in cattle.

Keywords

Cattle, vaccination, immune response, BoHV-4, genetic variability, systems immunology

Peripheral Blood Immunophenotyping in 50 dogs: comparison between healthy dogs and dogs with mast cell tumors

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Abstract

Mast cell tumors (MCT) are an important oncologic disease in dogs, representing the most common cutaneous neoplasms in this species. Like in humans, indolent and aggressive forms have been described, with variable behavior and patient survival times. The study of immune populations has gained relevance in cancer patients, but the phenotype of circulating immune cells in canine oncological cases is scarcely characterized. This study aimed at immunophenotyping blood leukocytes in dogs with MCT, in comparison with healthy dogs, expecting to contribute to the identification of new diagnostic/prognostic biomarkers. Blood samples were collected from 25 healthy and 25 dogs diagnosed with MCT. Frequency and phenotype of monocytes and T CD4⁺, CD8⁺, CD4⁺CD8⁺ lymphocytes were evaluated by flow cytometry. The distribution and phenotype of several of the analyzed blood immune populations were statistically different between cancer and healthy dogs. While the frequency of CD25-expressing cells was found increased in CD4 T cell MCT samples, cells expressing FoxP3 within that cell population were decreased. Contrarily, the frequency of FoxP3⁺ cells in the CD4⁺CD25⁺ population was increased in diseased dogs. The percentage of CD14⁺ monocytes was not different between groups, however the MHC class II median fluorescence intensity on these cells was significantly lower in cancer patients than in control dogs. This is the first study that characterizes the immunophenotype of circulating leukocytes in dogs affected by MCT. We expect to extend the analysis to a larger group of samples and to correlate the results with patients' outcomes.

CAC supported by FCT Grant 2021.08598.BD

Keywords

dog, mast cell tumors, blood leukocytes

Oxidative burst responsiveness as immune and stress parameter - comparison of chemiluminescence measurements of neutrophils' ROS production in different mammalian species

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Abstract

Introduction

Neutrophil granulocytes dispose of different antimicrobial mechanisms: besides phagocytosis and the formation of extracellular traps, they produce reactive oxygen species (ROS). Alterations in the quantity and activity of circulating immune cells are described as consequence of infection and stress, also having an impact on the neutrophils' oxidative capacity. Changes in the neutrophils' oxidative burst responsiveness can be used to assess an individual's health state.

We compared the ROS production of healthy individuals of different species to highlight species differences, but also to later distinguish the values of stressed or infected animals from those of healthy ones.

Methods

We use small blood samples (25-50µl) from six different species. With a portable luminometer the chemiluminescence resulting from the reaction of luminol and the ROS is measured. The values after stimulation with phorbol-12-myristate-13-acetate (PMA) are compared to an unstimulated control, values are taken every 10 minutes over a period of 90 minutes.

Results

Standard values of ROS production after secondary PMA stimulation were determined for six species ($n \geq 4$ each). The measured values are within a species-specific range, with small individual differences. The timepoint of maximum ROS production is similar within one species but shows notable interspecies differences.

Comparable results could be replicated using isolated neutrophils instead of whole blood.

Conclusion

The amount and time of maximum ROS production after stimulation with PMA is species-specific. In the future we aim to compare possibly stressed or infected animals with healthy individuals of the same species.

Keywords

Oxidative burst - Neutrophil Granulocytes - Chemiluminescence

Potential of IgG from spray dried porcine plasma (SDPP) to bind pathogens associated with canine enteropathies.

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Abstract

Spray dried porcine plasma (SDPP) contains immunomodulating proteins, most importantly immunoglobulins. These have been shown to increase the diversity of the gut microbiome of *e.g.* fish and mice. To discover whether SDPP's effects derive from its interaction with host pathogens, we studied whether IgG from SDPP is able to bind and affect propagation of canine-specific bacterial and viral pathogens, all involved in the development of canine chronic enteropathies (CE). To study the potential for SDPP to target canine pathogens, the levels of IgG, IgM and lactoferrin in SDPP were tested and found to be substantial, despite being heat sensitive: approx. 25%, 9% and >0.1% of total protein content. With a further focus on IgG, binding to 4 bacterial species, 2 strains of each species, associated with canine CE (*C. perfringens*, *E. coli*, *E. faecalis* & *S. canis*) was tested. IgG from SDPP was demonstrated to be able to bind all 4 bacterial species, even at protein concentrations as low as 10 µg/mL. Binding of these bacteria also resulted in a trend of bacteriostatic activity for a bacterial strain of *C. perfringens*, *E. coli* and *E. faecalis* and both strains of *S. canis*.

To conclude, porcine IgG in SDPP is able to bind various pathogens associated with canine enteropathies, thus forming a potentially beneficial addition to the diet of dogs suffering from CE.

Keywords

SDPP, canine chronic enteropathy, bacteriostatic effect

Existing acute *Ascaris* infection suppresses immune response against *Salmonella* infection in an *Ascaris-Salmonella* co-infected porcine model

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Abstract

Ascariasis, caused by the *Ascarid* round worm is highly prevalent in both humans and pig-farming resulting in significant morbidity and hence a major public health and economic concern. Also relevant to both humans and pig-farming is salmonellosis caused by the zoonotic bacteria, *Salmonella*. The aim of our study was to determine whether existing acute ascariasis impairs the effective immunological response against intestinal salmonellosis.

Pigs were grouped into four: Uninfected, single *Ascaris suum* and *Salmonella* Typhimurium infected and *Ascaris-salmonella* co-infected. Peripheral blood mononuclear cells were used for immunophenotyping and differential expression profiling using flow cytometry and bulk RNASeq respectively.

The *Ascaris* and co-infected pigs were observed to have a clear *Ascaris*-induced Th2 response compared to both the *Salmonella* infected and naïve groups whilst the *Salmonella* infected group was observed to have higher IL-17A+ frequencies compared to all groups. The *Salmonella* group had significant differentially expressed genes when compared to the *Ascaris* and co-infected groups. When compared to the naïve group, only the co-infected group was observed to have significant differentially expressed genes. Functional analysis using the KEGG database revealed activated TNF and IL-17 signalling pathways in the *Salmonella* group in comparison to the *Ascaris* group. However, these pathways were observed to be suppressed in the co-infected group in comparison to the *Salmonella* group.

Whilst *Ascaris* induced a Th2 response, *Salmonella* induced a Th1 response. Our data suggests an *Ascaris*-induced immunomodulation of responses against *Salmonella* in the co-infected group.

Keywords

Ascaris, salmonella, immunomodulation

Deciphering genetic factors of survival during PRRSV outbreaks

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Abstract

The objective of this study was to explore the ability of a panel of genetic markers and immunity parameters to predict the survival rates during a natural PRRSV outbreak. For this purpose, ten-week-old female Duroc pigs (n=129), obtained from 61 sows and 20 boars, were naturally infected with a highly pathogenic PRRSV genotype 1 strain. Prior to infection, piglets were screened for immunity parameters (IgG levels in plasma and *SOX13* mRNA expression in blood) and genetic markers associated to PRRSV immune response and innate and adaptive traits. Additionally, the 20 boars were genotyped with a panel of 133 single nucleotide polymorphisms (SNPs).

Survival analysis showed that the risk of dying was significantly higher for animals with low IgG levels in plasma and/or high *SOX13* mRNA expression in blood. The genotypes of the sires for SNPs associated with IgG plasma levels, CRP in serum, percentage of $\gamma\delta$ T cells, lymphocyte phagocytic capacity, total number of lymphocytes and leukocytes, mean corpuscular volume and mean corpuscular haemoglobin were significantly associated with the number of surviving offspring. Furthermore, SNPs located in *CD163* and *GBP5* genes were also associated to piglet survival. The effects of these SNPs were polygenic and cumulative: survival decreased from 96% to 11% as more susceptible alleles were accumulated for the different markers.

In conclusion, our results confirmed the existence of genetic variability in the susceptibility of pigs to PRRSV infection and provided a set of genetic markers associated with PRRSV survival.

Keywords

Pig, PRRSV, genetic markers

The impact of infection with the tissue-invasive intestinal nematode *Ascaris suum* on hepatic antiviral immunity

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Abstract

The intestinal helminth *Ascaris suum* and the hepatitis E virus (HEV) are widespread in pig production and pose a significant public health risk due to their zoonotic potential. Despite the widespread distribution of both pathogens in pigs and their inherent tropism for liver tissue, studies on the occurrence and consequences of coinfection are completely lacking.

Given that repeated *Ascaris* exposure leads to a dominant hepatic type 2 immune response, we hypothesized that concurrent *Ascaris* infection impairs hepatic antiviral immunity by compromising tissue integrity and counteracting type 1 immune circuits. Consequently, we assume a positive correlation between *Ascaris suum*-infected herds and HEV-positive animals, as well as an increase in viral load and pathology.

Fattening pigs (n=547) were sampled at the slaughterhouse. Individual pathological findings and origin data were also collected. Meat juice and serum samples were analyzed using ELISA to detect the presence of IgG Antibodies. Additionally, bile and liver tissue were analysed by qRT-PCR to quantify HEV RNA.

First analyses confirmed that acute ascariasis (~7 %) and HEV infections (~51 %) are prevalent, and that both pathogens co-occur in fattening pigs. However, no increased prevalence of HEV-exposed animals was observed in herds with acute *Ascariasis*. Additionally, no correlation was identified between the two infections and increased pathological findings.

This study provides for the first time, insight into the co-occurrence of *A. suum* and HEV infections in fattening pigs. However, further investigations are needed to understand the influence of simultaneous *Ascaris* infection on virus pathogenesis in the natural host pig.

Keywords

Co-infection; Host-pathogen-interaction

Protective effect against bovine neosporosis conferred by mucosal and subcutaneous immunisation with TLR agonists-adjuvanted *Neospora caninum* membrane antigens

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Abstract

The apicomplexan parasite *Neospora caninum* is a major causative agent of abortions and stillbirths in cattle, representing a global economic burden surpassing one billion dollars per year. Vaccination is considered the most cost-effective approach to manage neosporosis; however, no commercial vaccine is currently available to prevent this disease. We previously determined that mucosal immunisation with *N. caninum* membrane proteins plus CpG ODN adjuvant successfully protected mice challenged with this parasite by inducing a strong Th1-type response. Here, we aimed at improving the immunogenicity of the antigenic preparation for use in cattle. In that line, we included additional adjuvants to the preparation and promoted the host's systemic immune response by combining intranasal and subcutaneous dose administrations. Parasite-specific cellular and humoral responses were evaluated in immunised and sham-immunised Holstein-Friesian female calves. Immunisation raised *N. caninum*-specific serum IgG1 and IgG2, and saliva IgA antibodies. Moreover, it induced memory CD4+, CD8+, and TCRγδ T cells that proliferated extensively and produced IFN-gamma in response to antigenic restimulation. Protection conferred by immunisation was assessed in calves experimentally infected with *N. caninum*. Using nested PCR and multiple amplification replicas, we found that 8/8 control animals presented detectable brain parasitic DNA, while consistent parasitic DNA amplification occurred only in 3/8 brain samples from immunised calves. Taken together, these results show that our immunisation strategy was effective in inducing parasite-specific humoral and cellular immunity in the bovine host and encourage testing this strategy on a larger scale and in bovine pregnancy models.

Funded by FCT - PTDC/CVT-CVT/3045/2021.

Keywords

Neospora caninum, immunisation

Multi-antigenic vaccine strategies against *Rhipicephalus microplus* ticks

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Abstract

Rhipicephalus microplus infestations pose a significant challenge to global livestock, demanding sustainable control measures. Our group has developed a multi-antigen anti-tick vaccine that was ~78% protective against the cattle tick. However, such vaccine is commercially unfeasible since its production requires several fermentations. To address this problem, our group has been developing new anti-tick vaccines using two strategies: a) chimeric proteins combining full sequences of three different protective antigens each (trivalent), used by protein linkers; and b) multi-epitope single antigens comprising epitopes identified within ten protective antigens. Trivalent chimeric antigen sequences were cloned into plasmids and successfully produced in *E. coli* expression system, exhibiting purity and feasibility for scalable production. Regarding the multi-epitope vaccine strategy, we conducted extensive B and T-cell epitope mapping within the ten protective antigens. Peptide Microarray and Next-Generation Phage Display analyzes were performed in order to highlight linear and conformational B-cell epitopes, by interrogating the polyclonal sera from protected bovines. Using peptide microarrays, we identified six linear B-cell epitopes in 4/9 antigens. Additionally, one to three conformational B-cell epitopes were identified per antigen by Next-Generation Phage Display. Moreover, immunoinformatics analyses revealed 17 CD4 T-cell epitopes in 10/10 vaccine antigens, which covers seven BoLA-II alleles that are highly representative of South American Holstein-Friesian herds. Ultimately, these epitopes have been applied to the development of epitope-based chimeric antigens, which will undergo further screening for immunogenicity and efficacy. Our findings suggest potential for an easily producible, commercially viable anti-tick vaccine to combat *R. microplus* infestations in bovines. Support: FAPESP-2023/17939-0; 2023/04771-3.

Keywords

Anti-tick vaccine; *Rhipicephalus microplus*; Bovines.

Potential immunological biomarker for detection of *Mycobacterium bovis* infection in water buffalo: preliminary results

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Abstract

Bovine tuberculosis is a worldwide zoonosis that affects many species of domestic and wild animals, including water buffalo (*Bubalus bubalis*); *Mycobacterium bovis* is the main cause of infection.

We investigated the application of a MILLIPLEX[®] bovine cytokine/chemokine multiplex assay to identify candidate biomarkers of *M. bovis* infection in water buffalo.

Thirty-six buffaloes were divided into three groups based on ante-mortem and post-mortem tests: *M. bovis*-infected (IFN- γ test positive, visible post-mortem TB lesions; N = 11), *M. Bovis*-Suspected (IFN- γ test positive, no post-mortem TB lesions/no *M. bovis* detection; N = 14), and uninfected (Officially Tuberculosis-Free herds, IFN- γ test negative; N = 11).

Blood samples were stimulated *in vitro* with Phosphate-buffered saline (PBS), bovine PPD, Pokeweed Mitogen (PWM), respectively. 16-24h post-stimulation, plasma were collected. Levels of 14 cytokines (IFN- γ /IL-1 α /IL-1 β /IL-4/IL-6/IL-8/IL-10/IL-17A/MIP-1 α /IL-36RA/IP-10/MCP-1/MIP-1 β /TNF/VEGF-A) were evaluated using the MILLIPLEX[®] kit.

Cytokine's data were analyzed with GraphPad Prism 10.01 using an un-paired T-test or the non-parametric Mann-Whitney test.

Our results revealed that *M. bovis*-infected and *M. bovis*-suspected buffaloes release high levels of IFN- γ /IL-17/IL-1 α /IL-1 β /IL-6/IL-10/IP-10/MIP-1 α /MIP-1 β in response to PPDB stimulation, with statistically significant differences ($P \leq 0.05$) between PPDB-treated and PBS samples. No PPDB-specific release was instead observed for IL-4/VEGF-A/IL-36RA/MCP-1. TNF and IL-8 were outside the detection limit in several animals.

Comparative analyses between uninfected and *M. bovis*-infected and *M. bovis*-suspected groups showed a significantly lower levels ($P \leq 0.05$) in IFN- γ /IL-17/IL-1 α /IL-1 β /IL-6/IL-10/IP-10/MIP-1 α /MIP-1 β in uninfected animals in response to PPDB stimulation.

Our preliminary results suggest the potential use of several cytokines as biomarkers for the detection of *M. bovis* infection in water buffaloes.

Keywords

tuberculosis, buffalo, cytokines, biomarkers

Utilising cross-reactive transcription factor specific antibodies to extend the phenotyping of B cells in cattle.

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Abstract

The phenotyping of cattle B cells is hindered by the limited availability of cattle-specific reagents, such as anti-CD19 for analysis by flow cytometry. Cross-reactive transcription factor-specific antibodies have allowed the identification of B cell populations in other species, including pigs. A protein sequence alignment revealed high homology between key B cell markers including Pax5, Blimp1, IRF4, Bcl6 and B cell receptor component CD79 α across cattle, pigs, and humans. We hypothesised that cross-reactive antibodies could target these markers in flow cytometry, allowing for B cell identification. Our results suggest that total B cells can be identified by a CD79 α^{high} Pax5 $^{+}$ phenotype. Combined with markers previously used to characterise cattle B cells, we observed that many of these cells co-expressed IgL, CD40, CD20, CD21 and CD71. Additionally, a distinct Blimp1 $^{+}$ IRF4 $^{+}$ Pax5 $^{\text{dim}}$ population was observed, which resemble plasma cells. These cells were IgL $^{-}$ CD20 $^{-}$ but partially expressed CD71 and CD21, similar to porcine plasma cells. Furthermore, CD79 α^{+} Bcl6 $^{+}$ cells were identified in lymph nodes which may represent germinal centre B cells. Future work will focus on phenotyping antigen-specific B cells from cattle vaccinated with foot-and-mouth disease virus like particles. In addition, we will apply this extended B cell panel to different lymphoid organs, including Peyer's Patches to further validate these phenotypes.

Keywords

B cells, Flow Cytometry, Cattle, Phenotyping, Transcription Factors.

Simultaneous flow cytometric assay for phagocytosis, viability, and ROS production in leukocytes of rainbow trout (*Oncorhynchus mykiss*)

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Abstract

The aim of this study was to assess a simultaneous assay to evaluate phagocytosis, viability, and ROS production in *O. mykiss* leukocytes. Whole blood of six rainbow trout was collected and peripheral blood leukocytes (PBL) were purified by lysis of erythrocytes with water by inversion for 20s. The pHrodo™ Green *E. coli* Bioparticles® Conjugated and CellROX Deep Red Assay Kit (Thermo Fisher Scientific) were used for phagocytosis, and for cell viability and ROS production, respectively. PBLs ($1 \times 10^6/100\mu\text{L}$) were incubated with 20:1 particle:cell ratio of pHrodo™ Green *E. coli* for 2 hours at 16°C, after one wash with PBS 1% BSA, 500nM of CellROX® reagent was added for 30mins at RT. During the final 15mins 1μM of SYTOX® Blue Dead Cell was added. Cells were collected on a Cytoflex cytometer and data analyzed using Kaluza software (Beckman Coulter). This assay has the advantage of simultaneously determining cellular parameters by exploiting the multiparametric power of flow cytometry. The mean percentage and SD of the following populations was calculated: live (90.9 ± 3.7) and dead (9.1 ± 3.7) leukocytes; ROS⁺pHrodo⁻ (78.19 ± 4.67) and ROS⁺pHrodo⁺ (2.33 ± 0.98) live leukocytes; ROS⁺pHrodo⁻ (83.38 ± 4.56) and ROS⁺pHrodo⁺ (0.29 ± 0.10) live lymphoid cells and ROS⁺pHrodo⁻ (57.21 ± 7.46) and ROS⁺pHrodo⁺ (15.14 ± 2.10) live myeloid cells. In conclusion, this assay proved fast and effective for monitoring blood cellular functions in rainbow trout. Furthermore, our findings could pave the way for the use of this tool at farm level for health and welfare assessment in other fish species.

Keywords

flow cytometry, rainbow trout, phagocytosis

Novel long non-coding RNAs in ileocecal valve samples from Holstein cattle naturally infected with *Mycobacterium avium* subsp. *paratuberculosis*

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Abstract

Bovine paratuberculosis (PTB) is a chronic enteritis caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) that results in significant losses to the dairy industry worldwide. Long non-coding RNAs (lncRNAs) regulate gene expression but their functional understanding in relation to PTB is limited. In this study, lncRNAs were identified in ileocecal valve samples from control cows without lesions (N = 4), and with PTB-associated focal (N = 5) and diffuse (N = 5) lesions in intestinal tissues using RNA-Sequencing technology. The raw reads were uploaded into the CLC Bio Genomics Workbench and the trimmed reads were mapped to the *Bos taurus* ARS_UCD1.2.109 reference genome using the Large Gap Read Mapping tool. The resultant annotation allowed the identification of 728 lncRNAs using the FLExible Extraction of lncRNAs pipeline. lncRNAs differential expression (DE) analysis performed with *DESeq2* allowed the identification of 1, 6, and 2 DE lncRNAs in the comparisons of cows with focal lesion *versus* (vs) controls, diffuse lesion vs controls, and diffuse vs focal lesions, respectively. The DE lncRNAs were experimentally validated by RT-qPCR. Best DE lncRNAs partner analysis identified expression correlations between the lncRNA1086.1, lncRNA ENSBTAG00000050406, lncRNA_2340.1, and the *Inactive Phosphatidylinositol 3-Phosphatase 9* (*MTMR9*), *GM Domain Family member B* (*RGMB*), and the *homeobox A6* (*HOXA6*), respectively. The *MTMR9* negatively regulates DNA damage-induced apoptosis, the *RGMB* negatively regulates IL6 expression, and the *HOXA6* is a regulator of cell differentiation. The identified DE lncRNAs could allow the development of novel PTB diagnostic tools and have potential applications in breeding strategies for resistant cattle.

Keywords

lncRNAs, cattle, paratuberculosis

Similarities and differences in the cellular immune response between healthy and naturally *Mycobacterium bovis* infected water buffaloes and cattle

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Abstract

Bovine tuberculosis (BTB) is a disease affecting a wide range of animals, including the water buffalo (*Bubalus bubalis*). Because of its zoonotic importance and economic impact, it is considered a disease of global importance. Although buffaloes and cattle belong to the Bovidae family, they show significant differences on response to pathogens. Because of the paucity information on immune response of buffaloes to BTB infection, this study aims to compare antigen-specific cytokine-expressing cells in PBMC from buffaloes and cattle, respectively. Blood samples were collected from naturally-BTB infected, and free-BTB animals (18 and 19 buffaloes, 6 and 5 cattle, respectively). Whole blood was stimulated with PPD-B or PBS for 6 h, then an intracellular cytokine detection was performed by flow-cytometry. Principal component analysis of the dataset showed that responses between buffalo and cattle were different, with cattle having higher proportion of TNF- α ⁺, IFN- γ ⁺, IFN- γ ⁺IL-17⁺, IFN- γ ⁺IL-17⁻, CD4⁺IL-17⁺, CD4⁺IFN- γ ⁺, CD4⁺TNF- α ⁺, CD4⁺IFN- γ ⁺TNF- α ⁺, CD4⁺TNF- α ⁺IL-17⁺ cells in comparison to buffalo. According to their disease status, negative animals (both buffalo and cattle) were distinguished by the proportion of IFN- γ ⁺IL-17⁺, IL-17⁺, TNF- α ⁺IL-17⁺, TNF- α ⁺ and CD4⁺ cells. The PCA showed that there were few differences in the proportion of antigen-specific cells in blood stimulated or not with PPD-B. PPD-B stimulation revealed a significant increase in the proportion of CD4⁺TNF- α ⁺, CD4⁺IFN- γ ⁺IL-17⁻, CD4⁺IFN- γ ⁺IL-17⁺, CD4⁺TNF- α ⁺IL-17⁻ cells in BTB-infected buffalo in comparison to cattle. Our data might reflect the differences in response to *M. bovis* infection between buffaloes and cattle, however the implications on the pathogenesis of BTB in buffaloes are unknown.

Keywords

flow cytometry, tuberculosis, cytokines, ruminants

Unveiling Intestinal Cell Diversity: A Comprehensive Atlas of Chicken Enteroids and Breed-Specific Variances in Broilers and Layers

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Abstract

Understanding the avian gastrointestinal tract is crucial for the expanding global poultry industry, given its role as the primary gateway for both nutrients and pathogens. In this research, single cell transcriptomics was used to analyse chicken enteroids that develop optimally in suspension without the structural support required to produce mammalian enteroids, resulting in an inside-out enteroid conformation with media-facing apical brush borders and an inner core of lamina propria cells. We conducted a comprehensive analysis of 43,587 intestinal enteroid cell transcriptomes from two economically significant chicken breeds, broilers and layers, using the 10X Chromium platform. Through the investigation of conserved marker genes, we successfully categorized 21 distinct clusters, encompassing various cell types including enterocytes, Paneth cells, enteroendocrine cells (EECs), goblet cells, tuft cells, smooth muscle cells, fibroblasts, pericytes, telocytes, and glial cells, organized into five primary lineages: mesenchymal, epithelial, endothelial, immune, and neuronal. Notably, within the immune cell population, we observed significant heterogeneity, including macrophages, monocytes, heterophils, and multiple putative T cell subsets, including granulysin producing $\gamma\delta$ T cells. Significant heterogeneity was also observed within the hormone-secreting EECs, including candidate populations of enterochromaffin cells, L cells, D cells, I cells, K cells and X/M cells. Overall, this study of avian intestinal enteroid cellular composition provides insights that may be useful to enhance the regulation of feed efficiency and the development of disease control strategies in poultry by elucidating mechanisms underlying immunity and intestinal function.

Keywords

organoid, chicken, cell atlas

Activation profile of bovine T cells in healthy, *Mycobacterium avium* subsp. *paratuberculosis* naturally infected and Paratuberculosis affected cattle after Avium and Johnin PPDs stimulation

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Abstract

In cattle, the first defense event against *Mycobacterium avium* ssp *paratuberculosis* (MAP) infection consists in antigen capture and processing, followed by activation of cell-mediated immunoresponse. At first, naïve T cells differentiate into Thelper1 (Th1) secreting gamma interferon (IFN γ), activating macrophages. In the later stage, Thelper2 (Th2) cells secrete anti-inflammatory cytokines, down regulating Th1 cytokine secretion while promoting the humoral response.

In this survey, 18 cattle were classified into three phenotypic groups (healthy, MAP-infected, PTB-affected), based on outcomes of IFN- γ test, serological test and qPCR from feces. The main lymphocyte populations involved in the immune response to MAP were evaluated. Specifically, starting from the cell culture plates, used in the stimulation phase of IFN- γ test, the lymphocyte populations were assessed on the whole blood samples of each well stimulated with PBS, Av-PPD, J-PPD, PWM, respectively, after incubation overnight.

Lymphocyte subpopulations were labelled with CD3, CD4, CD8, IFN γ and T $\gamma\delta$ antibodies.

The CD4+/CD8+ ratio in healthy subjects remained constant over the four different stimulations. A significant up-regulation of CD4+ IFN γ secreting cells was observed in PTB-affected compared to healthy cattle. An increase in the CD8+ population was observed in healthy but MAP-infected animals, particularly after Av-PPD and J-PPD stimulation. The lymphocyte population of cytotoxic CD8+, deputed and programmed to remove the infecting bacterial cells, were predominant in the group of γ -reactive animals, indicative of an effective infection containment activity in the MAP-infected group that still not exhibiting seroconversion and MAP excretion with feces.

Research funded by Italian Ministry of Health RCIZSUM 06/2021.

Keywords

Paratuberculosis, T lymphocytes, Gamma-Interferon test

LEUKOGRAM OF COMMON BENT-WING BATS (*MINIOPTERUS SCHREIBERSII*) INFESTED WITH HEMOSPORIDIAN PARASITES

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Abstract

Hemosporidian parasites are known intraerythrocytic pathogens in humans and animals. Although they are carriers of viruses, bacteria and parasites, bats stay apparently healthy, with limiting self-damaging inflammatory response. The aim of this study was to estimate white blood cell (WBC) count and leukogram, in a population (n=16) of Common bent-wing bats (*Miniopterus schreibersii*), with hemosporidia detected on blood smears. For each animal, blood sample was drawn from the saphenous vein, into heparin-coated syringe, and blood smears were made. Romanowsky-stained smears were assessed microscopically at 40× HPF for estimation of WBC count (average number×2000), and at 100× HPF for percentage of different leukocyte types: neutrophils, lymphocytes – large, small, large granulated, and small granulated, monocytes, eosinophils and basophils. The smears were also used for evaluation of the level of parasitemia, based on the number hemosporidian macrogametocytes *per* field (100×). Semiquantitative evaluation of WBCs resulted in 6.7 (3.2-18.0) (median; minimum-maximum value) × 10⁶/mL, with following distribution: neutrophils 42.5 (19.0-67.0) %, lymphocytes 43 (17.0-70.0) %, monocytes 4.0 (1.0-8.0) %, eosinophils 9.0 (2.0-17.0) %, and basophils 0.0 (0.0-2.0) %. Among detected lymphocytes, large accounted for 12 (7.0-26.0) %, small 16.5 (5.0-51.0) %, large granulated 6.0 (0.0-17.0) %, and small granulated 2.0 (0.0-5.0) %. The number of hemosporidian macrogametocytes *per* field was 1.0 (0.0-4.0) at 500 detected red blood cells. The large range in total WBC count and differential formula possibly shows different immune responses that vary with age, the presence of other infections and parasites, and the level of stress response during capturing.

Keywords

blood smear, intraerythrocytic pathogens, leukocytes

Porcine bronchoalveolar lavage contains a unique distribution of immune cell phenotypes

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Abstract

Pigs are natural hosts for respiratory pathogens such as influenza A viruses, porcine reproductive and respiratory syndrome virus and porcine coronaviruses. The immune response to these pathogens originates in the respiratory tract, however, knowledge on the phenotype and distribution of lymphocytes in the respiratory tract of pigs is still limited. To gain insight into airway immune cell composition, lymphocytes were isolated from bronchoalveolar lavage (BAL), tracheobronchial lymph nodes (TBLN) but also blood of inbred Babraham pigs. T cells and B cells were analysed by a set of 10-13 colour flow cytometry panels. Additionally, TBLN and lung tissue were embedded in OCT compound for immunofluorescence imaging. The BAL revealed a large presence of activated $\gamma\delta$ T cells with a $CD8\alpha^+CD25^+ICOS^+$ phenotype while regulatory T cells ($CD4^+CD25^+Foxp3^+$) and T follicular helper cells ($CD4^+ICOS^+Bcl-6^+$) dominated in TBLN. Interestingly, very high frequencies of plasma cells $CD79\alpha^+Pax-5^{dim}IRF4^+Blimp-1^+$ were found in BAL compared to blood and TBLN, comprising between 40% to 60% of total B cells. However, germinal centre B cells ($CD79\alpha^+Pax-5^+Bcl-6^+$) were mainly present in TBLN. This study provides a detailed characterization of porcine T and B cell populations in BAL, revealing differences in comparison to secondary lymphoid tissues and blood. For spatial context, lung tissue sections will be stained with immunofluorescence protocols established on lymph node tissue to investigate the presence of ectopic germinal centres in the porcine lung after influenza infection. This will help elucidate how the local immune environment of the porcine lung changes in response to pulmonary infections.

Keywords

Pig, BAL, lymphocytes

The combined effect of genetics, gut microbiota, and environment on immunity in laying hens

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Abstract

Animals are vaccinated to protect them against various pathogens. However, vaccine responses can vary significantly due to several factors that can impact vaccine responses and/or immunity. To investigate this, we conducted a study on how genetics, microbiota, and rearing conditions affect vaccine responses and other immune parameters. We divided 400 chicks into eight groups to observe the influence of different hen lines (Rhode Island Red (RIR) and White Leghorn (LEG)), microbiota (by administering or not a cocktail of three antibiotics), and rearing conditions (whether or not they had access to an outdoor yard from 12 weeks until the end of the experiment). We monitored the humoral vaccine responses throughout the experiment by using ELISA for five different vaccines, and the cellular response was assessed by ELISpot (IFN- γ secretion after restimulating splenic immune cells). Other immune parameters measured included hematocrit levels, blood cell composition, immunoglobulins, and natural antibodies. Our study found that the RIR line had better vaccine responses than the LEG line, which even had non-responders. We also observed differences in blood cell composition between the lines. Microbiota perturbation altered vaccine responses and had an impact mainly on the LEG line, leading to a reduction in immunoglobulins and changes in blood cell composition. Rearing conditions also moderately affected vaccine responses and cell composition, particularly in the RIR line. In summary, our study highlights the influence of genetics, microbiota, and rearing conditions on vaccine responses and immune parameters that could be exploited to improve animal health.

Keywords

immunity, genetics, microbiota

Immunization of neonate piglets

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Abstract

Neonatal pigs have an unmaturing immune system, and responses to vaccination in the early days of life is not well studied. Here we investigated immune responses to variations of protein-based vaccines in suckling pigs.

Pigs vaccinated intramuscularly with 25 µg recombinant model antigen CTH522 at age 3, 10 or 17 days in CAF01 liposome adjuvant and revaccinated two weeks later responded to vaccination. An effect of age was observed as day 3 and day 10 pigs serum CTH522 IgG response increased 200-fold while day 17 pigs had a mean 1000-fold increase relative to sham vaccinated controls two weeks post boost. Serum CTH522 IgA responses were briefly increased at 7 days post boost with 3-, 10- and 20-fold responses relative to controls.

With an aim to induce immunity prior to weaning, a comparison between liposome CAF01 vs oil-in-water (o/w) CAF19 adjuvant showed significantly enhanced protein immunogenicity with o/w formulation in 3 days old piglets. This was evident in both serum IgG, IgA and IFN-γ release assay. There was no significant effect of dose titration from 0.25 mL-1 mL (dose from 25 µg to 100 µg) in CAF19 adjuvant. Vaccination with intra dermal immunization (IDAL air gun) of 25 µg protein in CAF01, CAF19 and TRIS buffer immunization confirmed the enhanced adjuvant effect of CAF19, but did not show any differences in immunogenicity between i.m. and i.d. immunization.

The current study clearly show that neonatal piglets are receptive to vaccination with generation of high antibody titers, but low CMI responses.

Keywords

neonatal pigs, adjuvants, vaccination

Comparative analysis of Equine MHC haplotypes in Austrian, German and Arabian horses using polymorphic microsatellites

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Abstract

It is known that the major histocompatibility complex (MHC) genes are the most polymorphic genes within the vertebrate genome and their gene products play an important role in innate and adaptive immune responses and may affect the development of autoimmune diseases and susceptibility or resistance to pathogens. In wild species, high polymorphism is maintained by balancing selection: (i) MHC heterozygotes are believed to be preferred over MHC homozygotes because they can present a wider range of antigens. (ii) Specific MHC alleles are favored that ensure protection against pathogens currently present in the environment. In the latter case, the preference for MHC changes over time together with the change in pathogen composition. In contrast, domestic animals undergo artificial selection. Thus, in addition to pathogen/fitness-related pressure, the pool of MHC genes present in domestic animals may be influenced by a human preference for specific traits. Hence, artificial selection, as performed in the case of domestic animals, may influence MHC diversity. This study aimed to investigate the polymorphism of MHC within and between different Austrian, German and Arabian horse breeds. We assessed the MHC diversity by determining the polymorphism of MHC microsatellites and MHC haplotypes. Use of the same MHC microsatellites allowed comparison of the results with those for previously tested horse populations. Additionally, the results presented here, pave the way to further analyze the associations and correlations between particular MHC haplotypes with the immune response against certain equine pathogens of interest (e.g., Equine Herpes Virus type 1, *Streptococcus equi*, Equine hepatitis virus).

Keywords

equine leukocyte antigen (ELA), equine pathogens, adaptive immunity

CD20 as new marker to define porcine B-cell subsets

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Abstract

In human and mice, CD20 is frequently used as pan B-cell marker in parallel to CD19, while downregulation of CD20 is associated with B-cell activation and plasma cell differentiation. We recently identified mAbs directed against porcine CD20 and in-depth characterization of porcine B cells in PBMC, as well as lymphatic and non-lymphatic organs was performed by flow cytometry. Up to 90% of CD79 α -defined porcine B cells co-expressed CD20 in all animals analyzed. While CD20 expression was more uniform in PBMC and lung, distinct CD20 dim and high populations were observed in spleen and lymph node. Within lymph node CD79 α ⁺ cells, up to five different B-cell subsets were identified based on their CD20/CD21 expression: CD20⁻CD21⁻, CD20⁻CD21⁺, CD20⁺CD21⁺, CD20^{+/hi}CD21^{hi}, and CD20^{hi}CD21^{+/dim}. Most abundant were CD20⁺CD21⁺ and CD20^{hi}CD21^{+/dim} phenotypes in all animals analyzed. Differential expression of co-stimulatory molecules CD80/86, as well as clear location of class-switched B cells (IgG⁺) could be assigned to distinct subsets. The first showed highest expression in CD20^{+/hi}CD21^{hi} B cells, the latter was located within the CD20^{hi}CD21^{+/dim} subset. Within the CD20^{hi}CD21^{+/dim} subset, highly proliferating B cells (Ki-67⁺) as well as Bcl-6⁺ B cells, characterizing germinal center B cells, were identified. Furthermore, recently introduced Blimp-1⁺IRF4⁺-defined porcine plasma cells in blood showed a clear downregulation of CD20 compared to less differentiated B cells. Our results highlight CD20 as valuable marker to identify porcine B cells. Different CD20-defined B-cell subsets were observed, pointing towards distinct activation and differentiation states.

Keywords

CD20, porcine, B cells

PD-1 as marker for porcine follicular T-helper cells

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Abstract

PD-1 (CD279) is an essential marker for the identification of exhausted T cells, but it is also expressed on a subset of T-follicular helper (Tfh) cells. Therefore, studying PD-1 would be beneficial for tracking and characterization of antigen-specific T cells following infection and/or vaccination. We tested the abundance of PD-1 transcripts in cells isolated from porcine lymph nodes (LN) by PrimeFlow™ RNA assay using PD-1 specific probes in combination with markers associated with a Tfh phenotype like ICOS and Bcl-6. PD-1 transcripts were found at elevated levels in ICOS⁺Bcl-6⁻, and especially ICOS⁺Bcl-6⁺ CD4⁺ T-cell subsets. Thus, confirming PD-1 as useful Tfh marker also in the pig. For an in-depth study of porcine Tfh cells and to further enable functional tests, anti-porcine PD-1 specific monoclonal antibodies (mAbs) were generated by mouse immunization. Anti-PD-1 mAbs stained a distinct population of CD4⁺ICOS⁺Bcl-6⁺ T cells in LN, while the marker was hardly present in PBMCs. Within the LN-derived Tfh cells, a small population co-expressing Foxp3 was observed, likely representing T-regulatory follicular cells (Tfr). In addition to the classical Tfh subset, a small PD-1 expressing population was observed in CD3⁺CD4⁻CD8^{high} expressing cells in LN. Ongoing experiments on sorted CD4⁺PD-1⁺ T cells from lymph node will confirm their Tfh function via activation of immunoglobulin production by B cells. In summary, PD-1 can be used as valid marker for porcine Tfh cells and enables future in-depth studies on this cell population in vaccination/infection settings.

Keywords

PD-1, T-follicular helper cells, swine

Immunomodulatory effects of a probiotic alone and upon vaccination against *Mycobacterium avium* subsp. *paratuberculosis*

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Abstract

Due to the lack of an effective treatment for paratuberculosis (PTB), caused by *Mycobacterium avium* subsp. *paratuberculosis* (Map), searching for alternative or complementary therapies is necessary. PTB has been linked to Inflammatory Bowel Disease (IBD), a condition for which probiotic treatment is promising. Probiotics have even shown to enhance vaccine-specific immunity in some diseases. In this work we have studied the effect of vaccination alone (CV: Map inactivated vaccine), a probiotic alone (PR: *Dietzia*) and both combined (CV-PR) prior to infection with Map in a rabbit model to evaluate protection and determine if *Dietzia* enhances vaccine immunity. Additionally, 16S rRNA sequencing and cytokine expression analysis have been performed to assess shifts in the microbiota and immune response in the gut associated lymphoid tissue (GALT). The probiotic alone decreased pro-inflammatory cytokines but increased Map burden in GALT showing it was detrimental to the host in the assayed conditions. CV and CV-PR presented similar protective parameters, although TNF and IFN- γ expression was lower in CV-PR. CV and CV-PR showed higher abundance compared to PR in *Christensenelleaceae* and *Butyricicoccus*, respectively, being both conditions linked to positive scenarios in IBD. Furthermore, CV-PR showed a higher abundance of *Butyricicoccus* compared to CV, as well. However, CV-PR showed higher abundance of *Burkholderiaceae* which is associated with IBD when compared to the control infected animals. These results indicate that although *Dietzia* positively changes the immunomicrobiological profile generated by vaccination to some extent, its benefits might not justify the administration of the combination since vaccine efficacy is not increased.

Keywords

probiotic, vaccination, microbiota

Yeast-based delivery for oral immunisation against *Eimeria tenella* in broiler chickens.

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Abstract

Eimeria tenella, a highly prevalent and economically significant protozoan parasite affecting poultry, poses challenges for control despite existing live parasite vaccines. An innovative approach for anticoccidial vaccination involves utilising the yeast *Saccharomyces cerevisiae* for both production and delivery of vaccine antigens. *Saccharomyces cerevisiae* is routinely included in chicken diets, serving as a source of protein as well as an immune booster. We developed a vaccine system based on *S. cerevisiae* surface display (pYD1) for oral delivery of *E. tenella* antigens, including Apical Membrane Antigen 1 (EtAMA1), Immune Mapped Protein 1 (EtIMP-1), and a single Microneme Protein 3 (EtMIC3) repeat. In an *in vivo* trial, mixed sex Cobb500 broiler chickens (n=28-32/group) were orally immunised with a mixture of pYD1-EtAMA1, pYD1-EtIMP1 and pYD1-EtMIC3. Empty yeast (pYD1 only) and unvaccinated groups were used as controls. Mean body weight gain post-challenge with a high dose of *E. tenella* (15,000 oocysts/animal) was significantly increased in vaccinated broiler chickens compared to mock-vaccinated controls. Conducting additional analyses of the immune response elicited in chickens will offer valuable understanding regarding the effectiveness of potential new vaccine platforms based on whole recombinant yeast and their ability to combat avian coccidiosis.

Keywords

Yeast-based oral vaccine - *Eimeria tenella* - Avian coccidiosis

Characterisation of immune responses in porcine precision-cut lymph node slices using TLR agonists and porcine circovirus 2

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Abstract

Pigs are a valuable source of protein for human nutrition and a large animal model for human developmental processes and diseases, due to their similarity in anatomy, genetics, immunology, and physiology. This has highlighted porcine immune research as a useful tool to investigate host immune responses against specific pathogens and to subsequently design more efficient vaccines for pigs and humans. However, different from mice and humans, porcine lymph nodes (LN) have an inverted structure that has been poorly investigated. The recent development of organ platforms allows *in vitro* assessment of immune responses and related physiological processes, while substantially reducing the number of animals necessary. Precision-cut tissue slices (PCTS) are 3D tissue explants of 100 to 600 µm thickness, derived from host organs and cultured *in vitro*, retaining the anatomical structures, metabolic functions, tissue homeostasis and immune responses. Within the on-going work, we phenotypically assessed in depth B cells derived from precision cut lymph node slices (PCLNS), following stimulation with TLR agonists and porcine circovirus 2 (PCV2). With the stimulations, B cells in PCLNS could differentiate into a plasma cell phenotype. Along with this, PCV2 specific antibody and cytokine responses in PCLNS were measured. The results so far indicate that PCLNS in pigs can generate PCV2 specific antibodies as well as an acute TNF- α , IL-10, IL-12 response, while maintaining their anatomical integrity for up to 5 days. Thus, PCLNS have great potential for an in-depth *in vitro* analysis of innate and adaptive immune responses along with understanding the LN anatomy.

Keywords

Precision-cut tissue slices, immune response, PCV2

The Contribution of Chicken Dendritic Cells to Vaccine-Mediated Immunity Against Infectious Bronchitis Virus

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Abstract

Infectious bronchitis virus (IBV) is an acute and highly contagious respiratory pathogen in chickens associated with decreased egg production. In the UK, IBV outbreaks lead to £24M/year in economic losses. It is unknown whether complications from IBV is related to its impact on immune cell functions. Dendritic cells (DC) are innate immune cells with central roles in the initiation and regulation of immune responses. During viral infection, DC play a pivotal role in stimulating CD8 T cell cytotoxic responses that are necessary for long-term immune protection. In chickens, the role of DC against IBV are unknown. Recently, the generation of the XCR1 induced ablation (XIA) chicken line has allowed researchers to examine the contributions of chicken XCR1⁺ DC to vaccine-mediated immunity (PMID:37954617). Two-week-old XIA^{+/-} chickens intravenously receives either vehicle control (n=40) or B/B drug (n=40) to induce DC ablation. Chickens were unvaccinated or vaccinated against IBV via the intranasal and intraocular routes 24 h later and 2 weeks later. At day 3 and 7 post-primary and -secondary vaccination, 5 chickens/group were assessed. RT-qPCR will determine viral load while flow cytometry will assess absolute immune cell numbers and cellular phenotypes and activation in the blood, spleen, lung, and trachea and their spatial distribution in the aforementioned tissues via immunofluorescence microscopy. Understanding the role of DC in chicken immune responses can help in developing better strategies for disease control.

Keywords

Chicken Dendritic Cells Vaccination

Vitamin D concentrations in cattle on farms with recurrent bovine tuberculosis and influence on immune gene and protein expression in response to tuberculin

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Abstract

A reservoir of bovine tuberculosis (bTB) is thought to remain in some herds with recurrent disease and therefore, investigating factors regulating immune responses may enhance understanding of disease pathogenesis and/or test reactivity. A minimum concentration of 30 ng/ml vitamin D (vit D) in serum is thought to offer immune benefits but many questions remain. This study aimed to evaluate the variation in circulating vit D concentrations in cattle from herds with recurrent bTB and assess their impact on the immune responses. Serum vit D concentration was measured by ELISA in blood samples collected from herds in Northern Ireland and the Republic of Ireland (n=9). A mean concentration of 31.92 ng/ml was detected, with 42% of cattle sampled (n=206) below the recommended threshold. Peripheral blood mononuclear cells from healthy cattle were then cultured in serum from pooled low (<20 ng/ml) and high (>50 ng/ml) vit D cattle groups and stimulated with 50 µg/ml *Mycobacterium bovis* purified protein derivative (PPDb). Although *IL1A*, *IL1B*, *IL10*, *IFNG*, *NOS2* and *IL8* were induced in response to PPDb, gene expression results were not significantly different between low and high vit D groups at 3 hours post-stimulation. IL-1β, IL-6 and IL-8 protein expression also showed induction, but the high vit D modulated IL8 expression ($P=0.059$) at 12 hours. In conclusion, this study identified potential vit D insufficiency and suggests that current circulating vit D concentrations are likely not sufficiently strong to influence innate cytokine expression, which may have bTB disease relevance.

Keywords

bovine TB, Vitamin D, Reservoir

Impact of an *Ascaris* infection on immune effector functions in *Salmonella* co-infected pigs

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Abstract

The roundworm *Ascaris* is one of the most widespread helminth infections, leading to chronic morbidity in humans and considerable economic losses in pig farming. In parallel, pigs are an important reservoir for the zoonotic Salmonellosis, where pigs can serve as asymptomatic carriers. Here, we asked whether an acute *Ascaris* infection influences the immune response to *Salmonella* in pigs. We observed higher bacterial burdens in experimentally coinfecting pigs compared to pigs infected with *Salmonella* alone. The impaired control of *Salmonella* in the coinfecting pigs was associated with repressed IFN- γ responses in the small intestine and with the alternative activation of gut macrophages evident in elevated CD206 expression. Additionally, macrophages from coinfecting pigs showed enhanced susceptibility to *Salmonella* infection *in vitro*. Further, *Ascaris* coinfection profoundly suppressed natural killer (NK) cell functionality. We observed completely abolished IL-12/IL-18 driven elevation of IFN- γ production in CD16⁺CD8 α ⁺perforin⁺ NK cells of *Salmonella* single-infected pigs. In line with impaired effector functions, NK cells from *Ascaris* single and coinfecting pigs displayed elevated expression of the NK cell inhibitory receptors (KLRA1 and NKG2A). In conclusion, our data indicate that an acute *Ascaris* infection modulates different immune effector functions with important negative consequences on the concurrent bacterial coinfection.

Keywords

Ascaris infection, macrophages, natural killer cells

Porcine neutrophil effector functions are impaired by the heat labile enterotoxin LT from enterotoxigenic *E. coli*

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Abstract

Enterotoxigenic *Escherichia coli* (ETEC) are a major cause of diarrheal illness in piglets. The enterotoxins, like LT and STa, produced by these pathogens result in a watery diarrhea. Despite the crucial role of neutrophils in combatting bacterial infections, our understanding of how enterotoxins impact neutrophil function is limited. To address this knowledge gap, we used purified heat-labile enterotoxin (LT) and synthesised heat-stable enterotoxin a (STa) to investigate their impact on the effector functions of neutrophils. Our study reveals that STa does not exert any discernible effect on the function of neutrophils. In contrast, the enterotoxin LT altered the migration and phagocytosis capacities of porcine neutrophils. In addition, LT induced the production of inflammatory factors and the release of neutrophil extracellular traps via activation of cAMP/PKA and ERK1/2 signalling in neutrophils. Our findings provide novel insights into the impact of LT on neutrophil function, shedding light on the underlying mechanisms that govern its immunoregulatory effects. This might help ETEC in subverting the immune system and establishing infection.

Keywords

neutrophils, *E. coli*, enterotoxin

Mapping early innate immune responses against African swine fever virus associated to lethality or immune protection

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Abstract

African swine fever (ASF) is a pandemic disease producing important worldwide economic and social consequences. The control of the disease, caused by the African swine fever virus (ASFV), is hampered due to the lack of effective vaccines and the poor knowledge on protective immunity. Up to date, live attenuated vaccines (LAV) are the only ones conferring solid protection, despite the associated biosafety concerns. However, the mechanisms associated with the LAV-induced protective immunity are not well characterized. To better understand the early innate immune events induced upon LAV vaccination, in this study we intranasally vaccinated pigs with the LAV Ba71ΔCD2. Temporal immune responses induced locally were compared to pigs lethally challenged with the parental ASFV BA71 strain. Virus dynamics across several lymphoid and non-lymphoid tissues collected at 0, 2-, 3-, 4-, and 7- days post-infection/vaccination were analysed by qPCR. Absolute numbers of the main immune cell subsets quantified by flow cytometry showed a clear distinction on their kinetics in challenged and vaccinated pigs. Indeed, pigs infected with the virulent virus showed a broad depletion of several immune cells, while in vaccinated pigs their numbers were maintained. Importantly, cytotoxic cells appeared as a distinctive marker of a favourable outcome in vaccinated animals. Altogether, these results contribute to a better understanding of the early protective immunity induced by an intranasal LAV, and thus reveal the immune sequential events to be targeted when developing ASF vaccines.

Keywords

ASFV, innate immunity and cytotoxic response

Immune Complex Induced Migration of Slan⁺ Non-Classical Monocytes and Its Implications in Systemic Lupus Erythematosus

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Abstract

The deposition of immune complexes (ICs) in tissues plays a key role in the immunopathogenesis of systemic lupus erythematosus (SLE). We have shown that ICs immobilized in the vasculature capture slan⁺ non-classical monocytes (slanMo) from blood flow and induce a haptokinetic response. This project now investigates a novel, IC-induced and gradient-directed migration mechanism of slanMo in the context of SLE. We utilized a transmigration assay to assess the chemotactic response of slanMo to different IC concentrations. Blocking experiments using antibodies to different Fcγ receptors (FcγRs) and pertussis toxin to block chemokine dependent migration demonstrated, that the induced migration is CD16 (FcγRIII) dependent. Furthermore, we compared the migration behaviour of slanMo with classical monocytes and other CD16-expressing cells, i.e. NK cells and neutrophils in response to different IC concentrations. Our findings show that IC induce a significant chemotactic effect on slanMo, comparable to known chemotactic agents such as C5a. This transmigration is specific to slanMo and is dependent on the concentration of IC. In contrast, classical monocytes and other CD16-expressing cells, including NK cells and neutrophils, did not exhibit an IC-dependent transmigration response. Further transcriptomic analysis and gene set enrichment analysis revealed a high enrichment score of a systemic lupus erythematosus gene set in slanMo responding to IC. The study concludes that slanMo exhibit a unique CD16-dependent migratory response to IC, distinguishing them from classical monocytes and other CD16-expressing cells. These findings highlight the specific role of slanMo in IC-mediated immune responses and support their role in the pathogenesis of SLE.

Keywords

monocytes, immune complexes, migration

Feline intestinal explant model to study interactions of *Toxoplasma gondii* with mucosal immune responses of its definitive host

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Abstract

Introduction : *Toxoplasma gondii* is considered the third most important of 31 foodborne parasites identified in Europe by the FAO/WHO, threatening public health. The development of effective vaccines or drugs against animal and human toxoplasmosis, has been hampered by a lack of data on the sexual reproductive cycle of *T. gondii* which occurs only in felids. Therefore, we aim to study the interactions of *T. gondii* with the feline intestinal microenvironment to decipher the local mucosal immune response and understand its impact on parasite life cycle.

Methods: Intestinal explants have previously been used to study intestinal pathologies in various species, but until now cat specific models are lacking. Here we report the development of a feline intestinal explant model including both duodenum and ileum tissues. Using this model, we applied RNAScope[®] technology on the intestinal tissue to study cytokine expression in different culture conditions and in presence or not of *T. gondii*.

Results : Explant enterocytes can be infected by *T. gondii* tachyzoites within 24h of infection *in vitro* in hyperoxic conditions and host cytokine responses can be detected. However, incubation in these hyperoxic conditions induced variations in both Th1 and Th2 cytokine expression.

Discussion/Conclusion : These preliminary results validate the use of intestinal explants and the RNAScope[®] technology to study *T. gondii* interactions with the feline intestinal mucosa and the immune response of the definitive host. A better understanding of these species-specific interactions will help develop vaccine strategies for improved animal and public health.

Keywords

Feline intestinal explants, *Toxoplasma gondii*, mucosal immune response

Natural extracts from grape marc influence response of porcine macrophages to lipopolysaccharide stimulation

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Abstract

The problem of antimicrobial resistance is one of the great challenges in animal production. The use of natural substances is one way how to improve the health of animals and thereby reduce the consumption of antibiotics. In our *in vitro* experiment, the immunomodulating effects of grape marc extracts with antimicrobial properties were tested. Porcine monocyte-derived macrophages (MDMs) were exposed to different concentrations of polyphenols extracted by different *Generally Recognised As Safe* solvents belonging to different chemical classes: glycol, ester, alcohol or ketone, to test cell viability and cytotoxicity. MDMs were then exposed to lipopolysaccharide either alone or in combination with polyphenols for 4 and 24 hours. Assessment of MDMs response was based on detection of relative expression of mRNA for selected pro- and anti-inflammatory cytokines. The cytotoxicity of polyphenols decreased with their decreasing concentration. Expression of mRNA changed more in relation with the solvent used to extract the polyphenols. Extract in alcohol and ester at concentrations under 2.5% and the 0.6%, respectively, was non-cytotoxic. These extracts at concentration of 0.6% led to slight or no change of mRNA expression of genes in comparison to levels in cells treated with LPS only. Extract in alcohol and ketone at concentration 5% and 0.6%, respectively, led to higher mRNA expression of genes of pro-inflammatory cytokines at 4 h. In addition, 0.6% extract in ketone led to higher mRNA expression of genes of anti-inflammatory cytokines. However, viability of cells treated with the extract were about 60 %. The work was supported by project NeoGiANT 101036768 (H2020-LC-GD-2020-4).

Keywords

polyphenols; macrophages; lipopolysaccharide

Different Immune Control of Gram-Positive and Gram-Negative Mammary Infections in Dairy Cows.

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Abstract

Mastitis represents one of the major concerns in dairy industry due to high production losses, therapy-associated and early cull costs, with relevant public health implications. Recent studies focused on mastitis resistance, but the mechanisms of susceptibility to intra-mammary infections still need to be clarified. Therefore, we investigated innate immunity in acellular bovine skim milk through cytofluorimetric analyses of bacterial killing activity against both Gram-positive and Gram-negative pathogens. Freshly cultured *E. coli* and *S. aureus* strains, coming from clinical cases of bovine mastitis, were incubated with colostrum and milk samples at different lactation time points from two groups of cows, purportedly representing mastitis-resistant and mastitis-susceptible breeds; bacterial cells were analyzed for vitality by flow cytometry following incorporation of vital dyes. N-acetyl- β -D-glucosaminidase (NAGase) activity was also investigated in milk and colostrum samples. Our findings revealed that colostrum and milk bacterial killing activity was mainly addressed against *S. aureus* compared to *E. coli*, with this activity correlated with milk NAGase levels. Furthermore, both killing of *S. aureus* and NAGase activity were negatively correlated to the elapsed time of lactation. Interestingly, samples from the allegedly mastitis-resistant breed displayed higher bacterial killing and NAGase activities. Our study confirms that different pathogens induce different immune responses in the host, including the mammary gland of dairy cows and contributes to a better understanding of the mechanisms of pathogenesis and immunoregulation during mastitis. This could help in the future definition of new diagnostic markers and new therapeutic protocols against specific pathogens.

Keywords

mastitis; bacterial killing activity; NAGase

Monocyte numbers and their phenotype differ between lactating and dry cows

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Abstract

Transition cows are susceptible to inflammatory diseases, including mastitis, endometritis, and metritis. This may be attributed to an altered functionality of monocyte-derived macrophages and dendritic cells that originate from differentially programmed circulating monocytes. The objective of this study was to analyze whether the lactating to dry cow transition affects key parameters of circulating monocytes and their subsets. The frequency of monocyte subpopulations (classical, cM CD14⁺CD16⁻; intermediate, intM CD14⁺CD16⁺; non-classical, ncM CD14⁻/CD16⁺) was found to be similar between L and D cows. D cows exhibited significantly lower cM counts/ml blood ($p < 0.02$) and in tendency lower ncM counts/ml blood ($p = 0.06$). L and D cow monocytes did not differ in CD14 and CD11a expression. However, CD11c expression was higher on L cow monocytes ($p < 0.01$). CD16 expression on D and L cow monocyte subpopulations differed significantly (higher on D cow ncM, $p < 0.001$; lower on D cow intM, $p < 0.05$). Heat-inactivated sera of D and L cows were analyzed for a range of different components and clustered separately after principal component analysis. Most significant differences ($p < 0.001$) were found for albumin, β hydroxybutyric acid, glucose, cholesterol, aspartate aminotransferase, and zinc. The number of blood monocyte subpopulations and the expression density of CD11c and CD16 on monocytes exhibited a significant correlation with zinc, glucose, and cholesterol levels. Collectively, these findings suggest the presence of transition period-associated processes, namely monocyte conversion, which may lead to the generation of monocyte-derived cells with altered functionality in vivo.

Keywords

Monocytes, Cattle, Transition period

A Comprehensive Pipeline to Rationalize T-cell Antigen Identification for Vaccine Development Against Complex Pathogens.

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Abstract

T-cells are vital for adaptive immunity, directly or by supporting B-cell responses. New vaccine technologies like mRNA emphasise selecting individual antigens as vaccine candidates. However, selecting T-cell antigens is challenging due to MHC diversity in host populations and the effect this has on the selection of peptides as epitopes. Information on MHC diversity in global livestock populations remains incomplete, however, the application of high-throughput technologies affords new opportunities to remove this obstacle to rational vaccine design rapidly. We have recently developed NGS-based BoLA-typing systems and applied this to cattle populations in countries including Brazil, Zambia, and UK, revealing a large repertoire of MHCI and MHCII alleles. By pairing this data with immunopeptidomic analysis, we have defined the peptide-binding motifs of a range of BoLA-I and BoLA-DR molecules and contributed to training MHC-binding prediction algorithms. Current work aims to i) sustain a fully-MHC defined (Holstein-Friesian) herd that can be used to support vaccine studies with the relevant immunoinformatic tools and ii) expand the application of immunoinformatics to cattle breeds that are important to livestock agriculture in LMICs. Combining immunoinformatic tools with pathogen data will contribute to a more rational approach to antigen selection for inducing T-cell-mediated immunity. Our current research focuses on *Theileria parva*, however the resources developed are applicable to a wide range of pathogens, and of particular value to complex pathogens where antigen identification remains a major bottleneck in vaccine development.

Keywords

T-cell, MHC, immunopeptidomic

Peripheral blood mononuclear cells co-cultured with extracellular vesicles secreted during an in vitro heat stress: Role on proliferative response

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Abstract

Extracellular vesicles (EVs) are released by various cell types, including immune cells in both normal and pathological conditions. During heat stress exposition, sheep exhibit a reduction of immune competence which enhances the susceptibility to inflammation. The aim of this study was to assess the biological role of the EVs produced during heat stress exposition in the sheep immune response. With this purpose, EVs released from sheep peripheral blood mononuclear cells (PBMCs) under in vitro heat stress (43°C) and normothermia (37°C) were tested for their role on the proliferative response. PBMC were isolated from peripheral blood of sheep (n=8), and a total of 2×10^6 cells were seeded into 96-well plate and co-cultured in presence of 10^{10} EVs for both 24 and 48h. Then, the colorimetric assay for assessing the cell metabolic activity (MTT) was performed. Cell proliferation index was affected by time ($P=0.0002$), in vitro EVs treatment ($P=0.04$), and their interaction ($P=0.008$). Particularly, PBMC registered higher proliferation index at 48h than 24h, and the proliferative index was higher in PBMC co-cultured with EVs from in vitro hyperthermia than the negative control (PBMC unstimulated). Furthermore, co-cultured PBMC with EVs from in vitro hyperthermia for 48h increased the proliferative index than the co-cultured PBMC with EVs from normothermia for 24h, the co-cultured PBMC with EVs from in vitro hyperthermia for 24h, and the negative control. Present data demonstrated the capability of EVs released during hyperthermia from PBMC of controlling the immune cell proliferation in a time-depended manner.

Keywords

Exosome, immune response, proliferation

Pathology of natural *Mycobacterium bovis* infection in alpacas

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Abstract

Introduction-Purpose:

Anatomo-histopathological features in alpacas naturally infected by *Mycobacterium bovis* are described.

Materials-Methods:

Alpacas necropsied within the Department of Agriculture, Food and the Marine - State Veterinary Laboratory (Ireland) from January 2010 to April 2024 were selected.

Results:

Tuberculosis (TB) was diagnosed in 71 alpacas (20 females, 29 males, 22 unrecorded) with a history of sudden death, weight loss, respiratory disease, and/or a positive ELISA test. Ages ranged from 3-month-old to 19-year-old (1 cria, 10 juveniles, 60 adults). Body condition score was good, moderate, poor or unrecorded in 24, 4, 16, and 27 animals, respectively.

Twelve animals (16.9%) had lesions on a single organ (11 lungs, 1 liver). Forty-six animals (64.8%) had lesions localized in both the lungs and liver. Thirty-three cases (46.5%) also had nodules/ulcers elsewhere (cranial lymph-nodes, kidneys, spleen, mesothelium), including unusual sites (trachea, pharynx, thyroid, uterus, intestine, skin). Pulmonary lesions had multiple presentations (from scattered miliary subpleural foci to caseous-calcified nodules up to 10x6x9cm, often cavitated). Hepatic lesions were often similar but lacked cavitation. Lymph-nodes were usually enlarged and frequently had caseous-chalky appearance on the cut surface. Histologically, nodular lesions were composed of multifocal to coalescing granulomas with a coagulative to liquefactive necrotic core, surrounded by variable number of epithelioid macrophages, multinucleated giant cells, fibroblasts, lympho-plasma cells, with scant to large numbers of ZN acid-alcohol fast bacilli.

Discussion-Conclusion:

TB lesions can be extensive despite non-specific symptoms. TB in camelids merit further studies.

To the authors' knowledge, this is the most extensive case-series TB pathology in alpacas.

Keywords

Alpaca; animal tuberculosis; necropsy; pathology; surveillance.

Metabolic fingerprinting of calves in response to BCG vaccination and experimental *Mycobacterium bovis* infection

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Abstract

Resistance to and intracellular persistence of *Mycobacterium bovis*, the cause of bovine TB (bTB) depends on the metabolic status of the host as well as the ability of the pathogen to exploit host nutrients, thereby determining disease outcomes. This study investigated the metabolomic changes in cattle before and after BCG vaccination, followed by experimental *M. bovis* infection, using advanced metabolomics profiling.

Blood serum samples from cattle (n=12) were collected pre- and post-vaccination with BCG Danish strain 1331 and after experimental *M. bovis* infection. Targeted high-throughput metabolite profiling was conducted using the Biocrates MxP[®] Quant 500 kit with UHPLC-MS. Metabolite concentration changes were analysed using paired univariant analysis implemented in Metaboanalyst software, with results adjusted for false discovery rate (FDR).

Preliminary analysis shows that BCG vaccination alone did not induce widespread metabolic alterations, with only one triglyceride (TG 18:1_30:2) showing significant variation ($|\log_2FC| \geq 1.5$, FDR < 0.05) between groups. However, the response to *M. bovis* infection revealed notable metabolic shifts. Specifically, nine cholesteryl esters (e.g., CE 20:5, CE 17:1) exhibited increased levels in the infected group, suggesting disrupted cholesterol metabolism. Additionally, there was decreased levels of four bile acids (e.g., CDCA, DCA) and 31 triglycerides (e.g., TG 18:2_36:3, TG 20:4_36:2) post-infection suggesting that *M. bovis* infection significantly alters lipid metabolism.

In addition, on-going work includes the analysis of comparisons with unvaccinated and experimentally infected calves which together with already generated results will add a new comprehensive layer of understanding to the metabolic impacts on the immune response to bTB.

Keywords

Metabolomics, bovine TB

