XIII Avian Immunology Research Group Meeting

ABSTRACT BOOK

Hosted by
the Roslin Institute,
the Royal (Dick) School of Veterinary Studies,
the University of Edinburgh

ROSLIN

THE UNIVERSITY of EDINBURGH
The Royal (Dick) School of Veterinary Studies

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AIRG 2026 Conference programme

Wednesday 2 September 2026 — The Roslin Institute

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Thursday 3 September 2026 — The Roslin Institute

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	Jim Kaufman *(Yale University, United States)*	Non-classical class II molecules in birds and mammals are induced by parasites
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19:00-23:00	**Social Event II South Hall Complex, Pollock Estate**	
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Friday 4 September 2026 — The Roslin Institute

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	Dominik von La Roche *(Ludwig-Maximilians-Universität München, Germany)*	Phenotypic characterization reveals tissue-specific features of chicken mucosal plasma cell populations	
	Johanna Trapp *(University of Veterinary Medicine Hannover, Germany)*	Do γδ T lymphocytes have an impact on the control of Infectious Bursal Disease Virus in the chicken gut?	
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	Oenone Bodman-Harris *(University of Surrey, UK)*	Receptor1(R1)-Targeted Antigen Delivery Vaccines Enhance Activation of Chicken HD11 Cells in vitro	
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Wednesday 2nd of September

Session I: Host-Pathogen Interactions I

Development of turkey primary intestinal organoids to model enteric viral pathogenesis and host innate immune responses

Jyothsna Girish[1], Matthew Quintanilla[1], Declan Kehlbeck[1], Alex Broadway[1], Megan Liu[1], Daniel Sung[1], Younngeon Jin[1], Kristen Diehl[2], Andrew Broadbent[1]

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Abstract

Avian enteric viruses cause major poultry losses, yet mechanisms underlying pathogenesis and host response remain poorly understood across different species, particularly in turkeys, where experimental resources are more limited than chickens. To address this, we developed turkey primary floating apical-out intestinal organoids and compared them with chicken cultures. Turkey and chicken organoids both contained epithelial cells, goblet cells, Paneth cells, and enteroendocrine cells. Bulk RNASeq revealed the cultures had similar transcriptomic profiles: 61% of genes were not differentially expressed between the two species, and of the remaining genes, no biological processes or pathways were significantly overrepresented. Using this system, we investigated two important enteric infections: avian reovirus (ARV) and low pathogenicity avian influenza virus (LPAIV). Both chicken and turkey organoids sustained ARV infection (strain S1133) with similar viral replication kinetics. Following infection, 685 genes were differentially expressed in turkey organoids and 469 in chicken at 8 hours post-infection (hpi). Of these, 133 were common between both species. ARV was associated with stress-adaptive metabolic reprogramming and altered ion transport in both species. Chicken cultures showed a stronger reduction in mature enterocyte transcripts, whereas turkey cultures showed stronger reductions in macrophage-like transcripts. Both chicken and turkey organoids also sustained LPAIV infection with strains A/turkey/Wisconsin/1966 (H9N2) and A/mallard/Minnesota/AI09-2749/2009 (H6N8). Viruses replicated to higher titers in turkey organoids compared to chicken, and RTqPCR analysis revealed Mx1 expression was higher in chicken organoids than turkey ($p<0.05$ at 24hpi with H9N2). In summary, turkey intestinal organoids are a useful platform to model avian enteric virus pathogenesis.

Temporal dynamics of conjunctival transcriptomic profiles during the house finch immune response to *Mycoplasma gallisepticum*

Enes Fahri Tezcan[1], Eleni Voukali[1], Ole Madsen[2], Marta Gòdia[2], Martin Těšický[3,4], Vladimír Beneš[5], Dana Hawley[6], Michal Vinkler[7]

[1]Department of Zoology, Faculty of Science, Charles University,, Prague, Czechia. [2]Animal Breeding and Genomics, Wageningen University & Research, Wageningen, Netherlands. [3]Faculty of Veterinary Medicine, Ludwig Maximilian University, Munich, Germany. [4]Institute of Vertebrate Biology, Czech Academy of Sciences, Brno, Czechia. [5]European Molecular Biology Laboratory (EMBL), Heidelberg, Germany. [6]Department of Biological Sciences,Virginia Tech, Blacksburg, United States. [7]Department of Zoology, Faculty of Science, Charles University, Prague, Czechia

Abstract

Emerging infectious diseases drive rapid shifts in host immune phenotypes. The bacterial pathogen *Mycoplasma gallisepticum* (MG) has recently adapted to a novel host, the house finch (Haemorhous mexicanus) showing consistently increasing virulence. This model host-pathogen system, a long co-evolutionary history resulting in the evolution of host tolerance to MG infection. This study investigates the temporal dynamics of house finch transcriptomic responses to MG. We focused on three distinct time points: 3, 6, and 13 days post-inoculation, representing the early, mid, and late phases of the response. At each time point, we compared MG-inoculated groups to sham-inoculated controls (60 wild-caught birds in total). To assess the effects of pathogen virulence on the immune response, we inoculated our treatment birds with either the VA1994 isolate (ancestral) or the NC2006 isolate (evolved). Transcriptomic profiles from conjunctiva and Harderian gland tissues were characterized via 3' mRNA-Seq.

Differential gene expression analyses revealed that MG-infected birds presented significantly altered transcriptomic profiles. The samples inoculated with the NC2006 strain showed enrichment for inflammatory pathways, whereas VA1994 induced a weaker response. The number of differentially expressed genes peaked at day 6 and declined by day 13, a pattern more pronounced in NC2006-treated birds compared to those inoculated with the VA1994 isolate. In this contribution, we focus on the dynamics of specific differentially expressed pathways. This research provides an in-depth study on the key transcriptomic insights behind the regulation of MG-induced inflammation, identifying putative genes and pathways for the mechanism of action of specific cell types during the response.

Innate Immune Reprogramming of Chicken Macrophages by Sodium Butyrate Enhances ROS-mediated Antimicrobial Responses *in vitro*

James R. G. Adams[1], Faisal R Anjum[2], Muhammad Yakubu Abare[2], Jai Mehat[1], Roberto La Ragione[1], Shahriar Behboudi[2]

[1]University of Surrey, Guildford, United Kingdom. [2]University of Bristol, Bristol, United Kingdom

Abstract

In the established model of classical trained immunity, metabolic and epigenetic hubs serve as central integrators of innate memory. While typically associated with pro-inflammatory reprogramming, the regulation of autophagy and cellular proteostasis remains essential for guiding macrophage differentiation and ensuring efficient pathogen clearance without excessive inflammation. In this study, we demonstrate that sodium butyrate (SB), a short-chain fatty acid, induces a functional profile that diverges from the canonical pathways observed in classical innate immune training. The induction of an innate reprogrammed state in chicken macrophages by SB is strictly dependent on the cellular developmental stage, occurring only during the early stages of differentiation from chicken bone marrow-derived macrophages but not in fully differentiated cells. This suggests that SB primarily facilitates an innate immune reprogramming with a specific temporal window of sensitivity. Our results show that SB-reprogrammed chicken macrophages exhibit enhanced reactive oxygen species (ROS) generation, altered cytokine expression, and an increased capacity to kill a diverse range of bacteria. Treatment with chemical inhibitors further demonstrated that these heightened antibacterial effects are directly attributed to increased ROS production and autophagy. In summary, these findings indicate that SB induces functional outcomes distinct from classical trained immunity and can elicit innate immune memory through alternative regulatory axes. Our data suggest that distinct innate reprogramming states

give rise to alternative activation programs and that innate immune memory exists along a spectrum of phenotypes rather than as a single, uniform state.

Changes in differential leucocyte counts and gastrointestinal microbiota composition associated with digestive and behavioural disorders in parrots

Kateřina Marková[1], Tereza Krajzingrová[1], Daniel Divín[1], Eleni Voukali[1], Martin Těšický[1,2], Veronika Grymová[3], Zuzana Knotková[4], Jakub Kreisinger[1], Michal Vinkler[1]

[1]Department of Zoology, Faculty of Science, Charles University, Prague, Czechia. [2]Ludwig Maximilian University, Faculty of Veterinary Medicine, Munich, Germany. [3]Veterinary clinics Avetum, Brno, Czechia. [4]Faculty of veterinary medicine, University of Veterinary Sciences, Brno, Czechia

Abstract

Parrots (Psittaciformes), an order of cognitively advanced birds, often suffer from behavioural disorders with depression-like symptoms analogous to humans (e.g., self-damaging behaviour, apathy, or anorexia). Growing body of evidence suggests that various mental health problems are associated with chronic neuroinflammation, which can result from unresolved peripheral inflammation. The 'leaky gut' hypothesis assumes that during gut microbiota dysbiosis, the permeability of the epithelial barrier increases, allowing microbial antigens to activate submucosal immune cells. Immune system mediates communication between gastro-intestinal tract and central nervous system through cytokines that can cross the blood-brain barrier and trigger gene expression changes directly in the brain. We have previously described the evolutionary loss of cannabinoid receptor CNR2 in parrots (an important regulator of neuroinflammation), which may lead to their higher sensitivity to neuroinflammation compared to passerines. We have also provided experimental evidence for increased systemic effects of peripheral inflammation on parrot brain gene expression. Now, in collaboration with veterinary practitioners, we have collected blood, faecal and oral samples of more than 400 individuals from 30 parrot genera. These included healthy individuals as well as those suffering from various digestive and behavioural disorders. We have analysed differential leucocyte counts in blood smears, a proxy to levels of peripheral inflammation. We have also analysed the composition of faecal and oral microbiota using 16S rRNA metabarcoding, showing that all the studied disorders were associated with decreased alpha diversity of gut microbiota. Here, the differences between healthy individuals and those suffering from digestive and behavioural disorders will be presented.

Session II: Innate Immunology

Comparative avian intestinal organoids reveal divergent host–pathogen responses during HPAIV H5N1 infection

Samantha Sives[1], Gabriel Euller-Nicolas[2], Rachel Busson[2], Chantal Allee[2], Paul Alun Brown[2], Beatrice Grasland[2], Lonneke Vervelde[3], Celine Courtillon[2], Kate Sutton[1]

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Abstract

Avian species display diverse responses to HPAIV infection, characterised by differences in viral replication and the magnitude and kinetics of host immune responses. Progress in understanding the mechanisms underlying these species-specific differences has been hindered by the limited availability of representative *in vitro* models, including comparable cell lines derived from the same tissue or cell lineage across multiple avian species. Avian intestinal organoids provide a physiologically relevant model for investigating species-specific host–pathogen interactions, retaining epithelial and lamina propria cell populations, whilst exhibiting apical-out polarity and extracellular matrix-independent growth. In this study, we generated intestinal organoids from chicken, turkey, and guinea fowl embryos to investigate HPAIV H5N1 replication and host immune responses at 1-, 24-, and 48-hour post-infection (hpi). H5N1 replicated efficiently in all three organoid models, producing infectious viral particles. Viral titres were significantly higher in turkey and guinea fowl organoids than in chicken organoids. At the host response level, interferon responses were significantly more pronounced in turkey organoids than in chicken or guinea fowl organoids. Transcriptomic analysis further revealed distinct species-specific response kinetics, with the greatest number of differentially expressed genes occurring at 1 hpi in chicken organoids. In contrast, peak transcriptional responses were observed at 48hpi in turkey and guinea fowl organoids. Collectively, these findings demonstrate that avian intestinal organoids provide a physiologically relevant platform for investigating species-specific differences in HPAIV replication and host immune responses.

The antiviral functions of avian orthologues of viperin against IAV

Miranda Nel[1,2], James Hodson[1], Nisha Kriplani,[1], Bo Wang[1], Kenneth Baillie[1], Jacqueline Smith[1], Rute Maria Pinto[1], Paul Digard[1]

[1]The Roslin Institute,, Edinburgh, United Kingdom. [2]University of Exeter, Penryn, United Kingdom

Abstract

Highly pathogenic forms of avian influenza A virus (IAV) typically cause severe disease in chickens but asymptomatic/mild disease in ducks. Previous transcriptomic studies have linked the interferon-stimulated gene RSAD2/viperin to this differential disease severity. Human viperin possesses antiviral activity in part through its radical-SAM domain. This domain catalyses the production of 3'-deoxy-3',4'- didehydro-CTP (ddhCTP), which acts as a chain terminator of flavivirus polymerases and inhibits global cellular translation.

We investigated the anti-IAV activity of chicken and duck viperin orthologues using ribonucleoprotein reconstitution assays (minireplicons). In mammalian and avian cell lines, both orthologues significantly reduced IAV gene expression, with the most pronounced inhibition caused by duck viperin. Despite

different strains of virus exhibiting varying sensitivity to viperin-mediated restriction, duck viperin consistently showed the greatest inhibitory effect.

We performed minireplicon assays using viperin orthologues with mutated radical-SAM domains or in the presence of ddhCTP, which suggested that viperin enzymatic activity is unlikely to contribute to the inhibition of IAV polymerase activity. Chicken and duck viperin were further shown to carry out radical-SAM-dependent repression of cellular protein synthesis, as previously shown for human viperin. However, no differences in this shutoff activity were observed between viperin orthologues.

Overall, we find that both chicken and duck viperin possess anti-IAV activity, which is not solely dependent on the enzymatic function nor the translational shutoff ability of the protein. Duck viperin consistently showed greater anti-IAV activity than its chicken counterpart, suggesting that it may indeed contribute to the greater resistance of ducks to IAV.

Metabolic regulation of chicken natural killer cell activation

Sayoko Maekawa[1,2], Daphne van Haarlem[1], Frank Vrieling[3], Rinke Stienstra[3], Christine Jansen[1]

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Abstract

Natural killer (NK) cells are innate immune cells that play an important role in antiviral immunity. In humans and mice, NK cell activation depends on metabolic reprogramming, and impaired metabolism is associated with reduced NK function. Little is known about the metabolic regulation of chicken NK cells. This study aimed to characterize the metabolism of chicken NK cells and its relationship with activation status.

Primary chicken NK cells were stimulated for 4 hours with PMA/Ionomycin or cultured for 48 hours in the presence or absence of recombinant chicken IL-2 +/- IL-15. NK cell activation (enhanced CD107 expression) and viability were assessed by flowcytometry. Metabolic activity was determined using CENCAT (Cellular Energetics through Non-Canonical Amino Acid Tagging), a recently-developed assay that measures metabolic activity in human immune cells.

Stimulation with PMA/Ionomycin resulted in an increase in glycolytic capacity compared to unstimulated cells (82.1±14.2% versus 41.5±12.8%), whilst the overall metabolic dependence on glucose did not change (84.75±6.1% versus 77.29±8.7%). The percentage of CD107+ cells increased significantly after PMA/Ionomycin stimulation (61.4±4.8% versus 22.4±2.1%). Stimulation with IL-2 +/- IL-15 did not induce changes in metabolic state, but the percentage of CD107+ cells was significantly higher in the cytokine-stimulated NK cells (33.7±+0.96% and 37.0±4.0%).

In conclusion, CENCAT enables analysis of chicken NK cell metabolism. Whereas strong NK cell activation was accompanied by increased glycolytic activation, milder activation induced no metabolic changes. These findings suggest that, also in chickens, metabolic reprogramming is linked to NK cell activation.

The 20E5 and 7C1 mAbs define functionally distinct chicken NK cell subsets

Sophia Melis, Daphne van Haarlem, Joost van Neerven, Chrisitne Jansen

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Abstract

Natural Killer (NK) cells play a pivotal role in antiviral-immunity. Mammalian NK cells are divided into NK1 and NK2 subsets, based on phenotype, function, and tissue localization. NK1 and NK2 are cytotoxic (NK1) or cytokine-producing (NK2). This study aimed to characterize the functional properties of distinct chicken NK cell subsets to study if they correspond to the NK1 and NK2 cells.

Splenocytes isolated from 14-day-old chicken embryos were stained with the mAbs 20E5, 5C7, 7C1, 17B12, and 21E3, that recognize chicken $CD3^{-}Bu\text{-}1^{-}IgL^{-}$NK-like cells. NK cell functionality was assessed by studying CD107 expression and the presence of intracellular perforin and IFN-γ. Correlations between CD107, perforin, and IFN-γ expression were evaluated by Spearman correlation analysis.

Perforin and IFN-γ were detected at similar levels in unstimulated and PMA/ionomycin-stimulated NK cells ($CD3^{-}Bu\text{-}1^{-}IgL^{-}$) . After stimulation the $20E5^{+}$ ($CD3^{-}Bu\text{-}1^{-}IgL^{-}20E5^{+}$) NK cells upregulated CD107 expression to higher levels compared to stimulated $7C1^{+}$ ($CD3^{-}Bu\text{-}1^{-}IgL^{-}7C1^{+}$) NK cells. In contrast, IFN-γ levels were only increased in the $7C1^{+}$ NK cells . Perforin levels did not differ between the subsets. However, perforin was positively correlated with CD107 expression in the $20E5^{+}$ subset, but negatively correlated in the $7C1^{+}$ subset.

These results suggest that whereas both NK subsets possess cytotoxic potential, $20E5^{+}$ NK cells have a greater degranulation potential, the $7C1^{+}$ NK cell population may represent a more cytokine-producing subset. To further confirm these findings qPCR analyses are performed to examine expression of NK1 and NK2 associated genes in the subsets.

A Self-Renewing Chicken Macrophage Line from Embryonic Liver for Modelling Tissue-Resident Innate Immunity

Faisal Anjum[1], Ayesha Siddiqua[1], Muhammad Yakubu Abare[1], Gyorgy Fejer[2], Shahriar Behboudi[1]

[1]University of Bristol, Bristol, United Kingdom. [2]University of Plymouth, Plymouth, United Kingdom

Title and authors included at the authors' request. The manuscript presenting these data has been submitted for publication.

What the Engineering Biology at Easter Bush (EB2) and the National Avian Research Facility (NARF) can offer to the Global Avian Research Community?

Samer Halabi

The Roslin Institute, Edinburgh, United Kingdom.

The increasing global demand for sustainable poultry production requires innovative approaches to improve bird health, welfare, disease resilience, and productivity. This presentation will showcase how the Engineering Biology Hub and the National Avian Research Facility (NARF) are creating a powerful platform for advancing avian science through the integration of engineering biology, genome technologies, and specialised research infrastructure.

The Roslin Engineering Biology Hub applies engineering principles to biological systems to accelerate the development of future farmed animals. Its research spans target discovery and validation, advanced in vitro models, reproductive technologies, and genome engineering. In avian species, these capabilities enable the identification and functional validation of genes associated with disease resistance, immune responses, welfare, and production traits, while facilitating the development of novel genome-editing and transgenic approaches.

Complementing these capabilities, NARF provides unique avian resources, specialist expertise, and research facilities that support studies in immunology, host–pathogen interactions, genome engineering, genetics, development, physiology, and behaviour. NARF maintains a number of poultry lines within both conventional and specified pathogen-free (SPF) facilities, providing essential resources for high-quality, reproducible research. These capabilities enable robust translational studies addressing major challenges facing the poultry sector, including infectious diseases, animal welfare, and sustainable production systems.

Together, the EB2 hub and NARF offer an end-to-end environment for discovery, validation, and application of innovative technologies in poultry research. This presentation will highlight current capabilities, ongoing research activities, and collaborative opportunities aimed at improving avian health and welfare while contributing to global food security and sustainable agriculture.

Session III: Host–Pathogen Interactions II

Chicken Three-Dimensional Intestinal Organoids as an *In Vitro* Model of Environmental Stress and Disease Resilience

Taylor Hanford[1,2], Samantha Sives[1], Salvatore Galgano[3,2], Mark Stevens[1], Lonneke Vervelde[4], Jos Houdijk[3,2], Kate Sutton[1,2]

[1]University of Edinburgh, Edinburgh, United Kingdom. [2]Centre for Tropical Livestock Genetics and Health, Edinburgh, United Kingdom. [3]Scotland's Rural College, Edinburgh, United Kingdom. [4]Royal GD, Deventer, Netherlands

Abstract

Resilience and disease resistance of indigenous poultry breeds, and their association with intestinal health, remain poorly characterised. *In vitro* models that have the potential to accurately assess intestinal responses to environmental and pathogenic stressors were lacking until the development of avian organoids. Avian organoids are *ex vivo* models that recapitulate key cellular components of the intestinal mucosa, including epithelial and lamina propria immune cell lineages. As such, they provide a physiologically relevant platform for investigating host–pathogen–environment interactions.

We aimed to develop an *in vitro* screening model using chicken intestinal organoids to characterise gut resilience to environmental stress and resistance to avian pathogenic *Escherichia coli* (APEC). APEC, a causative agent of avian colibacillosis that can colonise the gastrointestinal tract without causing clinical disease; however, environmental stressors, such as nutrient scarcity, may promote translocation to extra-intestinal sites, resulting in systemic infection. Organoids derived from broiler and layer embryos were cultured in media with decreasing dextrose concentrations to assess nutritional sensitivity and susceptibility to APEC infection and immune responses. APEC invasion, measured by CFU, peaked at 6 hpi and declined by 24 hpi, with no differences observed between breeds or dextrose

conditions. However, at mRNA level, genes associated with innate immunity, metabolism, and intestinal barrier function were differentially expressed following APEC infection and reduced dextrose exposure. In conclusion, chicken 3D intestinal organoids show potential as a screening platform for assessing host responses to environmental stressors and infection.

In ovo exposure to avian pathogenic *Escherichia coli* stimuli reduces splenic γδ T-cell frequencies in developing chicken embryos

Sina Bagheri, Dieter Liebhart

Poultry Medicine, Clinical Centre for Population Medicine in Fish, Pig, and Poultry, Clinical Department for Farm Animals and Food System Transformation, University of Veterinary Medicine Vienna, Vienna, Austria

Abstract

The chicken embryo represents a model for investigating early immune development and host–pathogen interactions in avian species. However, the capacity of the embryonic immune system to respond to bacterial antigens remains poorly understood. This study compared the immunological effects of live, gamma-irradiated, heat-killed, and formalin-killed avian pathogenic *Escherichia coli* (APEC), as well as lipopolysaccharide (LPS), on splenic immune responses in chicken embryos. Specific-pathogen-free layer embryos were inoculated on embryonic day 15 with the respective APEC/LPS preparations or PBS as a control. After three days of incubation, spleens were collected for flow cytometric immunophenotyping and RT-qPCR analyses. Individual spleen samples were analyzed for major immune cell populations, including $CD45^+$, $CD3^+$, $CD4^+$, $CD8\alpha^+$, $TCR\gamma\delta^+$, Bu-1^+ and $KUL01^+$ monocytes/macrophages. Expression of selected cytokine and Toll-like receptor genes was assessed by RT-qPCR. Inoculation with live APEC resulted in high mortality rates of the embryos, preventing further statistical analyses. Flow cytometry analysis demonstrated that gamma-irradiated APEC and LPS induced comparable immunological responses with a significant reduction in splenic $TCR\gamma\delta^+$ frequencies. RT-qPCR showed that gamma-irradiated APEC and LPS induced comparable responses, characterized by increased expression of *IFN-γ* and *IL-1β*. In contrast, heat-killed and formalin-killed APEC preparations elicited only minor responses and did not differ significantly from control embryos. The findings demonstrate that bacterial antigens can induce measurable cellular and molecular immune responses during embryonic development and specifically the involvement of embryonic $TCR\gamma\delta^+$. Therefore, the results offer new insights into the mechanisms of embryonic immune activation and the impact of antigen preparation on variability.

Mapping Chicken Immune Cell Landscapes During MDV-Induced Tumorigenesis

Yaoyao Zhang, Lily Yang, Shaozhi Zuo, Weicheng Li, Liping Liu, Graham Freimanis, Tim Downing, Venugopal Nair, Yongxiu Yao

The Pirbright Institute, Woking, United Kingdom

Abstract

Marek's disease virus (MDV) is a highly oncogenic alphaherpesvirus that induces T-cell lymphoma in susceptible chickens. Advances in transcriptomic technologies have provided valuable insights into MDV-associated oncogenic pathways, viral pathogenicity, and host responses. However, the cellular

heterogeneity of the immune response and the stepwise processes underlying MDV-induced tumorigenesis remain incompletely understood. To characterise immune cell populations and their responses to MDV infection at single-cell resolution, we performed single-cell RNA sequencing (scRNA-seq) on tumour cells isolated from MDV-infected chickens and splenocytes from mock-infected controls.

Computational analysis identified 22 distinct cell clusters, which were primarily annotated as B cells and T cells based on the expression of established marker genes. Comparative analysis between tumour and control samples revealed marked changes in immune cell composition and cluster distribution following MDV infection. In addition, viral transcript mapping enabled the detection and quantification of MDV RNA expression at the single-cell level, allowing the identification of virus-positive cell populations and assessment of viral transcriptional activity across different immune cell subsets. Differential gene expression and pathway analyses further characterised the molecular changes associated with infection and tumour development.

Together, these analyses provide a high-resolution atlas of the chicken immune landscape during MDV-induced tumorigenesis, revealing both host transcriptional reprogramming and viral gene expression at single-cell resolution. This work provides a valuable resource for identifying MDV target cell populations and advances our understanding of the cellular mechanisms underlying oncogenic viral infection.

Thursday 3rd of September

Session IV: Adaptive Immunology I & Vaccines I

scRNA-seq Reveals Cytolytic and Interferon Stimulated γδ T Cells in TCR Cβ Knockout Chickens

Theresa von Heyl[1], Milena Brunner[1], Antonia Schmauser[1], Christine Wurmser[2], Simon Früh[3], Benjamin Schusser[1]

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Abstract

Generating T cell receptor (TCR) knockout (KO) chickens has provided a crucial tool to investigate T cell biology in birds. While the knockout of γδ T cells results in compensation through CD8+ αβ T cells in the gut, the loss of αβ T cells leads to a severe phenotype, including granuloma formation in the skin, inflammation in the stomach and spleen, and impaired development of lymphatic organs such as the thymus and bursa. However, despite these advances, the in-depth characterization of T cell subsets in chickens remains challenging, primarily due to the lack of specific markers to differentiate classical Th1, Th2, and Th17 populations. The advent of single-cell RNA sequencing (scRNA-seq) provides a powerful approach to overcome these limitations and enables detailed resolution of T cell heterogeneity.

To gain deeper insights into the functions and characteristics of αβ and γδ T cell subsets in chickens, whole transcriptome analysis was performed on TCR Cβ KO, TCR Cγ KO, and WT chickens from embryonic day 18, days 3 and 14 post-hatch. The results revealed that γδ T cells exhibit cytolytic activity in both TCR Cβ KO and WT chickens - a function absent in TCR Cγ KO chickens. Furthermore, the severe phenotype observed in TCR Cβ KO chickens was associated with a distinct cluster of γδ T cells expressing genes linked to the interferon pathway. These findings provide new insights into chicken T cell biology and improve our understanding of the mechanisms underlying the severe phenotype observed in TCR Cβ KO chickens.

Analyzing the role of S1PR1, CCR7 and PECAM-1 during chicken B cell development

Sabrina Schleibinger[1], Maria Vogt[1], Camila Gatti Corrêa[1], Theresa M. Pauli[1], Milena Brunner[1], Theresa von Heyl[1], Benjamin Schusser[1,2]

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Abstract

In chickens, B cell development is initiated during embryogenesis within the bursa of Fabricius, a primary lymphoid organ unique to birds. Hematopoietic progenitors migrate towards the bursa during embryonic development and undergo proliferation and immunoglobulin gene rearrangement within the microenvironment of the bursa. The precise orchestration of B cell migration to and within the bursa

is critical for establishing functional B cell compartments. However, the molecular mechanisms regulating these migratory processes during embryogenesis remain incompletely understood.

Recent transcriptome analysis of chicken B cells identified the genes *S1PR1, CCR7* and *PECAM-1* as potential regulators of B lymphocyte migration during embryonic development. By delivering CRISPR/Cas9 ribonucleoprotein complexes into the developing embryo utilizing virus-like particles (VLPs) it became feasible to analyze the role of these genes *in vivo* during B cell development. The VLPs targeting the genes of interest were injected intravenously on embryonic day (ED) 12, when most of the precursor B cells were still located in the spleen. Subsequently the migration of B cells towards the bursa of Fabricius was analyzed by flow cytometry. Editing efficiencies of 20% were detected in the bursocytes and a knock down of the target genes resulted in significantly reduced B cell numbers in the bursa compared to the control group. In conclusion, this study shows that S1PR1, CCR7 and PECAM-1 play a significant role in the migration of developing B cells in the chicken embryo.

Transcriptomic and immunophenotypic characterization of dendritic cells and macrophages in the avian bursa of Fabricius

Emoke Szocs[1], Stefania Zsofia Bogya[1], Bettina Kaczur[1], Adam Soos[1], Viktoria Halasy[1], Samantha Sives[2], Sonja Hartle[3], Kate Sutton[2], Nandor Nagy[1]

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Abstract

The bursa of Fabricius (BF) is an avian primary lymphoid organ essential for B-cell maturation. Within BF follicles, reticular epithelial cells and bursal secretory dendritic cells (BSDCs) provide a specialized microenvironment for B-cell development. However, the ontogeny, immunophenotype, and functions of BSDCs remain incompletely understood. This study aimed to characterize these myeloid populations, identify selective membrane markers for BSDCs, and elucidate their developmental fate. We demonstrate that colony-stimulating factor 1 receptor (CSF1R) selectively labels BSDCs across embryonic and adult stages. Using *CSF1R*-eGFP transgenic chickens, we show that CSF1R+ BSDCs co-express CD11d, p75-neurotrophin receptor, and alphaV/beta3 integrin. Immunoelectron microscopy confirmed that medullary CSF1R+/p75+ BSDCs and TIM4+/Lamp1+ macrophages represent morphologically distinct populations. Based on this immunophenotypic characterization, we isolated $CSF1R^{high}/TIM4^{neg}$ medullary myeloid cells (BSDCs) and cortical $CSF1R^{low}/TIM4^{pos}$ myeloid cells (macrophages) via fluorescence-activated cell sorting. Subsequent RNA sequencing and differential gene expression analysis revealed distinct transcriptomic profiles, highlighting *CXCL13* as a key BSDC signature gene. We validate this using RNAscope *in situ* hybridization, confirming that immigrating CSF1R+ BSDCs express *CXCL13* following colonization of the follicular primordia to recruit CXCR5+ B-cell precursors. Furthermore, *in vitro* assays using embryonic bursal explants demonstrate that CXCL13 induces bursal B-cell emigration.

In conclusion, identification of selective BSDC markers and transcriptomic signatures allows their efficient isolation and molecular characterization, and reveals that vimentin+/CSF1R+/CD11+/p75+ BSDCs provide a CXCL13-dependent niche to recruit and organize developing B cells. These findings highlight BSDCs as active regulators of B-cell maturation and support the importance of dendritic-cell-derived factors in primary lymphoid organ development.

When Genetics Matter: Vaccination and Infection Responses in Local and Crossbred Chickens

Luise Freier[1], Inga Tiemann[2], Josefine Stuff[2], Steffen Weigend[3], Maryna Kuryshko[1], Elsayed M. Abdelwhab[1], Ulrike Blohm[1]

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Abstract

Chickens are an important livestock species worldwide. Intensive selection has improved the performance of commercial stocks but displaced numerous local breeds, reducing genetic diversity. In contrast, local breeds, although less productive, may retain valuable traits such as enhanced disease resilience and are therefore relevant for sustainable breeding. Crossbreeding with commercial lines may help balance productivity and genetic diversity. Here, three German local breeds (Altsteirer, Bielefelder, Ramelsloher) and their crossbreeds with high-performing layers were evaluated for vaccine response and disease resilience.

Humoral and cellular immune responses to Newcastle disease virus (NDV) vaccination were assessed using a prime-boost regimen. All animals developed NDV-specific antibodies after a single dose, with crossbreeds showing higher titers than local breeds. Booster vaccination enhanced humoral responses in all local breeds, most prominently in Altsteirer. Clear breed-specific differences were observed in cellular responses after in vitro re-stimulation: Altsteirer showed enhanced responses after boosting, whereas Bielefelder exhibited reduced T-cell proliferative capacity after the second dose. In crossbreeds, these differences were minimal and no reduced proliferative capacity was observed.

Infection with an avian influenza model virus revealed further breed-specific differences. Ramelsloher displayed the mildest clinical disease and highest survival. In Ramelsloher and Bielefelder, enhanced pulmonary cytotoxic T-cell infiltration at 4 dpi correlated with reduced oropharyngeal virus shedding at 7 dpi. Remarkably, transmission to sentinel chickens was observed exclusively in Altsteirer. Studies investigating infection dynamics in crossbreed chickens are ongoing.

Overall, these findings demonstrate distinct breed-specific immune profiles and highlight the potential of integrating local genetic resources into sustainable poultry breeding.

Broad cross-protection against IBDV enabled by an inactivated vaccine incorporating two antigenically distinct strains

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Abstract

Infectious bursal disease virus (IBDV) remains a major threat to poultry production worldwide due to its immunosuppressive effects and ongoing antigenic diversification, which limit the efficacy of conventional vaccines. Here, we evaluated the immunogenicity and cross-protective efficacy of an inactivated multivalent vaccine containing the classical IBDV strain GB02 and antigenic variant strain 89/03. Using both specific pathogen-free (SPF) and commercial chickens, the vaccine was

administered either alone or within prime–boost regimens and assessed for antibody responses, maternal antibody transfer, and protection against homologous and heterologous challenges.

Vaccination induced robust and sustained virus-neutralizing antibody responses against both classical and variant IBDV strains, persisting for at least 100 weeks of age in parental birds. High levels of maternally derived antibodies (MDAs) were efficiently transferred to progeny and remained detectable for at least four weeks post-hatch. In laboratory challenge studies, progeny of vaccinated birds were protected against classical and very virulent strains and showed markedly enhanced protection against antigenic variants when derived from prime–boost vaccinated hens. Under field conditions, consistent serological responses and ≥90% protection against diverse IBDV strains were confirmed in commercial flocks.

These findings demonstrate that inclusion of both classical and variant IBDV antigens in an inactivated, multivalent formulation (Nobilis Multriva™) enables broad and durable protection across genetically diverse IBDV strains. This strategy effectively addresses limitations of existing inactivated IBDV-containing vaccines and supports improved control of IBD in both breeder and progeny populations.

Early-life immune phenotyping identifies candidate biomarkers of immunocompetence associated with broad vaccine responsiveness in laying hens

Abdullah Al Momen Sabuj, Nicolas Bruneau, Catherine Denis, Alice Racanati, Fanny Calenge, Marie-Hélène Pinard-van der Laan, Fany Blanc

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Abstract

Assessing immunocompetence in healthy animals remains a major challenge for improving vaccination strategies and breeding disease-resilient poultry. Although immune responses following vaccination have been extensively studied, little is known about whether early-life immune phenotypes are associated with responsiveness to multiple vaccines.

Approximately 400 commercial laying hens were immune-phenotyped at 5 weeks of age using a panel including hematology, plasma absorbance profiles, serum protein electrophoresis, acute-phase proteins, immunoglobulins, vitamin D concentration and blood leukocyte immunophenotyping. Birds subsequently received the routine commercial vaccination program. Humoral responses to four viral vaccines were evaluated at 22 weeks of age and integrated into a composite vaccine responsiveness score. Newcastle disease virus (NDV)-specific cellular immunity was assessed by IFN-γ ELISpot. Univariate and multivariate analyses were used to identify immune traits associated with vaccine responsiveness.

Preliminary analyses identified distinct early-life immune signatures associated with vaccine responsiveness. Associations were strongest for humoral responses and varied across vaccines, suggesting both shared and antigen-specific determinants. In contrast, correlates of NDV-specific cellular immunity differed, indicating that humoral and cellular responsiveness may rely on partially distinct immune traits. Multivariate analyses further supported these findings by identifying combinations of immune parameters associated with response profiles.

These findings show that early-life immune phenotyping can identify candidate biomarkers of immunocompetence associated with broad vaccine responsiveness and provide a framework for

integrating immune phenotypes with genomic information to support precision vaccination and genetic improvement of poultry.

MVA-H5: A Promising DIVA-Compatible Vaccine Platform for Birds Against Highly Pathogenic Avian Influenza

Rebecca Dressler[1], Flora Fischer[2], Mareen Vogler[3], Dominik von La Roche[1], Monika Rinder[3], Robert Fux[2], Sonja Härtle[1]

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Abstract

The emergence of a novel H5N1 clade shifted epidemiology of highly pathogenic avian influenza (HPAIV) in Europe from seasonal, migration driven toward an endemic circulation, significantly impacting poultry production and wildlife. Current control measures—biosecurity, non-vaccination policies, and traditional outbreak response—are increasingly ineffective, costly, and socially challenged, underscoring the need for complementary strategies such as vaccination. However, effective vaccines must support surveillance, requiring DIVA compatibility. Modified Vaccinia virus Ankara (MVA), originally developed for human smallpox vaccination, is a safe and well-characterized viral vector in mammalian vaccine research against diverse infectious diseases, but data on its applicability in birds remain limited.

Using homologous DNA recombination, we generated a recombinant MVA (MVA-H5) expressing the H5-antigen of a recent European HPAIV isolate. In vitro replication capacity was assessed in chicken embryo fibroblast (CEF) cultures. Chickens were vaccinated using different routes of administration to evaluate viral biodistribution and dissemination kinetics by qPCR and the immune responses by a flow cytometry based white blood differential, ELISA and Elispot assays.

Despite efficient *in vitro* replication, the vaccine virus showed only limited, sporadic, and transient detection beyond the application site *in vivo*. Correspondingly, a distinct innate and adaptive immune cell response in blood was observed following intramuscular vaccination, but not after oral or ocular administration. First analysis of serum samples from intramuscularly vaccinated birds demonstrated a strong IgM and IgY response for H5 and MVA.

These initial findings suggest that MVA-H5 is a promising candidate as a safe and effective HPAIV vaccine for diverse avian species.

Evolution of the bursa of Fabricius from secondary lymphoid tissue.

Michael Ratcliffe

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Abstract

There are fundamental differences between avian species and human/primates in the pathways by which B lymphocytes develop and antibody repertoires are generated. In human/primates, similar to most mammals, B cell development occurs in the bone marrow and the stages of development are regulated by the expression of various components of the B cell receptor for antigen, surface immunoglobulin. In contrast, avian B cell development occurs in a specialized organ, the bursa of Fabricius, which is absolutely required for the creation of a normal diversified peripheral B cell compartment. Similarly, in most mammals the repertoire of B cell specificities is generated by the process of Immunoglobulin gene rearrangement involving selection from multiple functional variable, diversity and joining sequences, whereas in contrast, avian B cell repertoires are generated by a process of somatic gene conversion that is fundamentally distinct from the process of immunoglobulin gene rearrangement. Despite these differences, overarching similarities in B cell development suggest that these two systems evolved from a common ancestral model rather than evolving completely independent of one another. A likely scenario by which the constraints surrounding the evolution of avian species have driven the evolution of avian B cell development from a pre-existing multi-VDJ model of B cell development will be discussed.

Session VI: Genetics, Genomics, Evolution & Emerging Tools

Divergent and convergent evolution in avian TLR4 receptor system

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Abstract

During co-evolution with pathogens, host immune systems diversify through continual arms races. This process is evident at the interspecific level as adaptive divergence in immune genes and their protein products. However, in response to similar selective pressures, different species may also evolve similar protein features, resulting in molecular convergence. Birds, owing to their broad ecological diversity and the availability of compact, high-quality genomes, provide an excellent system for exploring how positive selection shapes immune gene variation. We focus on divergent and convergent molecular patterns in two selected TLR4-associated immune receptor complexes: TLR4–MD2 and CD180–MD1. First, we identified the distribution of positively selected sites within the proteins. These sites frequently occurred at ligand-binding and dimerization interfaces, suggesting functional adaptations that fine-tune pathogen-recognition mechanisms. Next, using 3D protein modelling, we analysed divergence and convergence in surface electrostatic potential patterns. We established a novel approach that restricts protein surface analysis to functionally important regions, thereby increasing the sensitivity of molecular-convergence detection. Surface-charge phenotypes formed clusters that were independent of phylogenetic relationships, indicating multiple instances of convergent evolution in receptor phenotypes across avian taxa. The resulting patterns of amino-acid variation in immune genes were highly complex. Overall, our findings highlight convergent evolution as an important force shaping immune innovation.

Genomic variability and structural organization of the chicken IgH D-gene locus

Milena Brunner[1], Johannes Geibel[2], Tom V. L. Berghof[1], Annett Weigend[2], Steffen Weigend[2], Benjamin Schusser[1]

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Abstract

Antibody diversification in chickens differs fundamentally from mammals, as the chicken immunoglobulin heavy chain (IgH) locus contains only a single functional V- and J-gene segment, but approximately fifteen D-segments were identified. Consequently, D-genes represent the primary source of combinatorial diversity generated through germline V(D)J-recombination in chickens. Despite its unique role in shaping the antibody repertoire, the genomic organization and variability of chicken D-genes remain poorly understood, and the locus is still largely unresolved in current reference genomes.

To overcome these limitations, a targeted long-range amplification strategy and a custom bioinformatic analysis pipeline were developed to analyze the highly repetitive chicken D-gene locus. Using amplicon sequencing, the genomic D-gene region was analyzed across a diverse set of genetically distinct chicken lines. Comparative analyses revealed that the organization of the D-gene locus is conserved across chicken lines, characterized by a recurrent D1-Dx-D2 core cluster followed by additional D-genes assigned to D3-D15. Despite this conserved structure, substantial variation in D-gene content and sequence diversity was observed between individual animals and lines. In addition, D-gene usage within rearranged IgH repertoires was analyzed and compared to the corresponding germline configuration. This revealed lineage-specific differences in D-gene usage and indicated that certain germline D-gene variants are preferentially rearranged, while locus organization and sequence-specific characteristics may contribute to differential D-gene rearrangement frequencies.

Together, these findings provide new insights into the genomic architecture and variability of the chicken IgH D-gene locus and improve our understanding of how germline D-gene diversity contributes to antibody repertoire formation.

Dissecting the Harderian Gland Immune Response to Avian Influenza Using Single-Cell Multiomics

Ying Wang[1], Liqi An[1], Susan Lamont[2], Rodrigo Gallardo[1], Huaijun Zhou[1]

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Abstract

Avian influenza (AI) remains a major threat to poultry production and public health. Host genetics influences susceptibility and immune responses, making selective breeding a promising strategy to enhance disease resistance. The Harderian gland (HG), a key mucosal immune organ in chickens, plays an important role in defense against avian influenza virus (AIV). This study investigated the genetic and cellular mechanisms underlying resistance to low pathogenic avian influenza (LPAI) in the HG.

Three-week-old Fayoumi (resistant) and Leghorn (susceptible) chickens were challenged with H6N2 LPAIV (10^7 EID_{50}). Viral load, AIV-specific antibody responses, inflammatory responses, and HG immune cell populations were evaluated. At 4 days post-infection (dpi), HG samples from three birds per treatment group (n = 12) were analyzed using 10x Genomics single-cell Multiome (RNA + ATAC) sequencing, generating an average of >250 million reads per library.

Infected Leghorns exhibited significantly higher viral titers and lower macrophage frequencies than Fayoumis at both 1 and 4 dpi, indicating a less effective early innate immune response. Conversely,

Leghorns produced higher AIV-specific antibody levels at 7 and 14 dpi, suggesting a delayed but stronger adaptive humoral response. The HG single-cell Multiome dataset contained 118,031 high-quality cells. Viral transcript abundance was greater in infected Leghorns, consistent with RT-qPCR results. Integrated transcriptomic and chromatin accessibility analyses identified seven major cell types and nine immune subclusters, including multiple T- and B-cell populations. Ongoing analyses will define the molecular pathways regulating genetic resistance to AI and support precision breeding strategies to improve disease resistance and vaccine efficacy.

Neuropeptide expression links to cytokine levels during acute and chronic peripheral inflammation and associates with behaviour in parrots

Daniel Divín, Eleni Voukali, Kateřina Marková, Nithya Kuttiyarthu Veetil, Balraj Melepat, Martin Těšický, Tao Li, Michal Vinkler

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Abstract

Chronic inflammation is recognized as a key factor in the development of numerous mental disorders, including depression or anxiety. Research in humans and laboratory rodents has shown that bidirectional communication between the immune and nervous systems allows peripheral inflammation to influence neural functions. In this context, neuropeptides are now studied as important modulators of neuroinflammation. Immune system does not function as an isolated entity but as part of an integrated regulatory network involving the nervous and endocrine systems. Among birds, parrots provide a unique model for studying neuroimmune interactions. These cognitively advanced birds are highly susceptible to behavioural disorders, including depression-like behaviour, feather plucking, and anorexia. We have previously shown that parrots have lost the cannabinoid receptor 2 gene (*CNR2*), a key immunomodulatory receptor associated with a neuroinflammatory response, suggesting altered communication between the immune and the central nervous system. Here, we present our research targeted on neuroimmune crosstalk in parrots, focusing on interactions between cytokines and neuropeptides during experimentally induced inflammation. Using transcriptomic and targeted gene expression approaches, we investigate the regulation of cytokines (IL1B, IL6, IL10, IL18, CXCLi2) and neuropeptides (NPY, PDYN, VIP, POMC, TAC1) in peripheral tissues and the brain during acute and chronic inflammation. Attention is also paid to the associations between neuroimmune signalling and behavioural variation. The findings highlight the tight integration of neural and immune regulatory pathways in parrots and demonstrate the value of investigation of not only classical immune mediators, but also molecules traditionally associated with other physiological systems in immunity.

Identification of novel antiviral immune genes in a new T2T chicken genome

Grace Schmidt, Tae Hyun Kim

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Abstract

The immune gene repertoire of avians is significantly smaller than that of mammals. In chickens, key antiviral regulators are believed to be missing from the genome, despite the chicken's ability to mount a

robust antiviral response. Because of this, the molecular mechanisms that mediate antiviral immune responses in chickens have yet to be fully understood.

Telomere-to-telomere (T2T) sequencing technology has allowed for the gapless assembly of genomes with regions that are difficult to sequence. Recently, a T2T chicken genome assembly was published (Ggswu) with 39.4Mb of newly resolved regions compared to Galgal6. These previously unresolved regions are gene-rich and may host missing immune genes. Therefore, the objective of this study was to search for novel immune gene candidates in the chicken T2T genome.

To compare the gene annotations of the new Ggswu to the current GRCg7b reference, each gene model was generated using the Eukaryotic Gene Annotation Pipeline (EGAPx) utilizing FAANG transcriptomes from ten chicken tissues. To identify novel coding regions in Ggswu, the predicted proteomes were compared using OrthoFinder, resulting in the identification of 911 unique protein-coding regions.

Furthermore, we aligned transcriptomic data from LPAIV-infected chicken tissues against Ggswu and 33 of the unique protein-coding regions were differentially expressed (padj < 0.05). Pfam-based HMMER searches identified zinc-finger, RNA-recognition, and DNA-binding motifs in the novel differentially expressed genes, suggesting potential transcription factor activity. Our preliminary findings suggest that some previously "missing" immune genes may be present in these newly resolved genomic regions and warrant further functional investigation.

Non-classical class II molecules in birds and mammals are induced by parasites

Aimee Parker[1], Tina Dalgaard[2], Andrew MacDonald[3], Karsten Skjodt[4], Jim Kaufman[5,3]

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Abstract

The chicken BF-BL region is compact and simple compared to the MHC of typical mammals. Similar to chicken classical class I genes, there are two class II B genes, of which only BLB2 is widely- and strongly-expressed. The other class II B gene, BLB1, is poorly expressed systemically. There are also two dedicated chaperone genes, of which DMB2 is widely- and strongly-expressed. The other chaperone gene, DMB1, is strongly expressed by RT-PCR only in intestinal tissues (and very much less well-expressed in thymus and brain). This tissue distribution has also been reported for the single duck DMB1 gene. Antibodies to the cytoplasmic tails showed chicken DMB2 expressed in cells of the lamina propria with DMB1 expressed in intestinal epithelial cells. RT-PCR of highly-purified intestinal epithelial cells showed RNA expression of both DMB1 and DMB2, but western blot showed only DMB1 protein, suggesting control after transcription. Upon repeating the original experiments, no expression of DMB1 was found in the intestine, and analysis after various treatments showed that DMB1 is upregulated upon exposure to helminth worms, but not several viruses, bacteria or a single-celled parasite. Published tissue staining shows more chicken CD4+ intraepithelial lymphocytes after nematode worm infection, the MHC specificity of which would be interesting to know. Moreover, bulk RNAseq of mouse ileum shows upregulation of Ebeta2 and Mb2 after schistosome infection, and human Langerhans cells are reported to express DQ2 molecules. Such lineage- and tissue-specific class II molecules may represent ancient modes of defense against metazoan parasites.

Developing Functional Readouts to Investigate Trained Immunity in Chicken Monocytes

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Abstract

Trained immunity is defined as a memory-like characteristic of the innate immune system. Even though recent studies indicate that trained immunity can be induced in chicken monocytes, the underlying mechanisms remain unknown. This is mainly due to the limited availability of readouts to measure trained immunity in chickens. Therefore, this study aimed to develop and identify functional readouts to enable mechanistic studies of trained immunity in chicken monocytes.

PBMCs were isolated from heparinized blood of Ross308 chickens and used directly or cryopreserved for later use. KUL01$^+$ monocytes were enriched using MACS and exposed to a variety of training agents for 24h. On day 6, the monocytes were restimulated with LPS for 24h. Readouts included IL-6 production (ELISA), nitric oxide (NO) production (Griess assay), surface expression of the activation markers CD40 and MHC-II (flow cytometry), and cellular metabolism (CENCAT).

The novel readout IL-6 production increased 1.4- to 18-fold (N=5) upon training and paralleled the increase in NO production (1.2- to 4.8-fold, N=5). This increase in NO and IL-6 was only observed when freshly isolated cells were used. In these experiments, CD40 expression increased upon LPS stimulation (1.3-fold, N=5) but was not affected by training, consistent with previous studies on trained immunity in broilers. MHC-II expression was not affected.

These results identify IL-6 and NO production as potential readouts to measure trained immunity in chicken monocytes. Building on these functional readouts, ongoing studies are exploring metabolic reprogramming as a potential underlying mechanism of trained immunity in chickens.

In vitro organoid model of leaky gut syndrome

Jeff Ling[1], Spiridoula Athanasiadou[2], Neil Mabbott[1], Lonneke Vervelde[3], Kate Sutton[1]

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Abstract

Necrotic enteritis, an enteric infection in chickens caused by NetB-positive *Clostridium perfringens*, represents a major economic threat to the global poultry industry, costing approximately $6 billion annually. The disease frequently manifests sub-clinically as increased intestinal permeability ('leaky gut'), leading to impaired nutrient absorption and increased feed-conversion ratio. Following the ban of antibiotic growth promoters and given the absence of effective commercial vaccines, novel interventions such as feed additives are increasingly necessary to control this disease. To model 'leaky gut' syndrome *in vitro*, we utilised chicken 3D intestinal organoids with a unique apical-out conformation containing epithelial and tissue-resident immune cells. We developed a high-throughput barrier integrity assay where organoids were treated with EDTA to induce barrier permeability, followed by the addition of 40 kDa FITC dextran. To accurately quantify barrier disruption via FITC permeation into the organoid core, an optimised image processing pipeline using the machine learning platforms Cellpose and CellProfiler was developed. This computational approach identifies individual organoids and normalises

target fluorescence data relative to organoid size, ensuring increased accuracy. Currently, this platform is being utilised to investigate the bioactivity of recombinant NetB protein, a key virulence factor of avian necrotic enteritis. Ultimately, this model offers a valuable tool for evaluating novel feed additives and biological compounds aimed at restoring, enhancing, or maintaining epithelial barrier integrity against necrotic enteritis.

Friday 4th September

Session VII: Adaptive Immunology II

Characterization of bursa-infiltrating T cells following IBDV vaccination in chickens

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Abstract

Infectious Bursal Disease Virus (IBDV) is a major pathogen that causes disease and immunosuppression in chickens. IBDV primarily targets B cells in the bursa of Fabricius (BF). Following infection, large numbers of αβ and γδ T cells accumulate in the BF; however, the composition and specificity of these T cell populations remain uncharacterized.

The aim of this study was to characterize IBDV-induced T cells at the clonal level and identify virus-reactive clonotypes. We employed next-generation sequencing (NGS)-based TCR profiling in combination with flow cytometry to analyze the TCR repertoire after vaccination and infection, both at the tissue level and within sorted T cell subpopulations.

Flow cytometry and TCR profiling revealed substantial inter-individual variability in the T cell response to IBDV. T cells in the BF following vaccination or infection were diverse and largely resembled peripheral blood or splenic repertoires in non-vaccinated chickens, suggesting non-specific recruitment of T cells. Notably, $CD4^+$ αβ T cells from vaccinated animals – but not $CD8^+$ T cells or γδ T cells – exhibited consistently larger clone group sizes among the most frequent clonotypes. This pattern suggests moderate, antigen-driven clonal expansion of T helper cells and enabled the identification of potential IBDV-specific CDR3 motifs.

Together, these findings demonstrate that IBDV induces a diverse T cell response in the BF, with clonal dynamics that differ between individuals and T cell subsets. This work highlights the complexity of T cell responses to viral infection in chickens and underscores the importance of clonal repertoire analysis for analyzing T cell immunity.

Phenotypic characterization reveals tissue-specific features of chicken mucosal plasma cell populations

Dominik von La Roche[1], Nandor Nagy[2], Sonja Härtle[1]

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Abstract

Plasma cells (PCs) are key mediators of humoral immunity, yet their characterization in chickens remained limited by the lack of suitable markers. Using the Harderian gland (HG), we previously identified a FSC^{high}/$CD45^{med}$/$chB6^{neg}$/sIg^{pos}/$CD57^{pos}$/$CXCR4^{high}$/$TACI^{low}$ cell population. These cells showed microscopic characteristics of plasmablasts (PBs) and PCs and high abundance of RNA for the PC transcription factor BLIMP1. The cells retained surface B-cell receptor (BCR) expression and a large

proportion of these cells had undergone class-switch recombination to IgA. In addition, we identified two cross-reacting antibodies for IRF4, a master regulator of PC differentiation, which allows for the specific identification of PBs/PCs by flow cytometry. Immunohistological analyses revealed abundant IRF4-positive cells throughout both the head and body regions of the HG, demonstrating a very broad distribution of PCs within the gland.

Analysis of cells from the ileal lamina propria identified a FSC^{high}/$CD45^{pos}$/sIg^{pos}/$CD57^{pos}$ population with a phenotype partially resembling that of HG PBs/PCs, including the loss of the characteristic B-cell markers chB6 and BAFF-R. These cells were mostly surface IgA positive, displayed strong BLIMP1 and IRF4 expression and were therefore considered as intestinal PCs. Interestingly, they differed markedly from HG PBs/PCs as in contrast to HG PB/PCs, intestinal PCs retained substantial MHCII expression, showed little or no TACI expression and lacked the pronounced CXCR4 up- and CXC5 downregulation, indicating dependence on different survival and migration signals.

Together, these findings establish novel tools for the identification of chicken PCs and provide evidence for distinct plasma-cell phenotypes in different mucosal tissues.

Do γδ T lymphocytes have an impact on the control of Infectious Bursal Disease Virus in the chicken gut?

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Abstract

The infectious bursal disease virus (IBDV) induces immunosuppression in chickens. IBDV enters the bloodstream via the cecum. In chickens, high numbers of γδ T cells are detected in the intestinal lining, but the contribution of γδ T cells to the immune response after IBDV infection has not been further defined. We used a γδ T cell knockout (KO) chicken model to elucidate the impact of γδ T cells on IBDV pathogenesis. At three weeks of age, groups of wildtype (WT) and KO birds were either inoculated with a placebo, an intermediate-plus (iIBDV; experiment, E1, E2) or very virulent strain of IBDV (vvIBDV; E3) and were sacrificed at three days post inoculation. Higher IBDV antigen loads were observed in the bursa of Fabricius, caecum and caecal tonsils of vvIBDV-infected chickens compared to iIBDV-inoculated birds. Also, differences in αβ T cell numbers, macrophages and circulating monocytes were detected between the three experiments. Overall, the IBDV strain impacted the gut-associated IBDV pathogenesis. As KO birds showed higher viral loads by RT-qPCR compared to the WT chickens for both viral strains. γδ T cells seem to contribute to the control of virus replication. We may speculate that depletion of the investigated immune cell populations in the cecum is a result of re-localization to the bursa of Fabricius.

Session VIII: Vaccines II

Effect of dietary biochar on broiler chickens challenged with *E. tenella* infection

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Abstract

Eimeria infections (coccidiosis) constitute a major problem in poultry husbandry and current control measures, chemoprophylaxis and live vaccines, are unsustainable. Sustainable alternative control strategies are needed, and it has been suggested that biochar has anticoccidial potentials. This study investigated whether dietary biochar could inhibit *Eimeria* invasion of the gut and potentially influence the caecal microbiota as well as immune responses of broiler chickens infected with *Eimeria tenella*. A total of 64 chicks were randomly allocated to one of two dietary treatments; control or biochar. Half of each group were inoculated *E. tenella* at 20 days old. The results show that dietary biochar did not inhibit the *E. tenella* invasion in the caecum, shown by comparable infection outcomes such as oocyst output, lesion scores, and growth rate between the control and biochar groups. Furthermore, biochar did not significantly affect blood leukocyte counts or induction of parasite specific immunity assessed as IFN-γ production and T cell activation (blast transformation and expression of CD25) in PBMC cultures. However, biochar induced a reduction in potential beneficial bacterial families such as *Lactobacillaceae* and *Bifidobacteriaceae* and an increase in the fungal family *Aspergillaceae* in the caecum. These alterations of the microbiome were similar to those induced by the *E. tenella* infection. Thus, our results suggest that dietary supplementation with biochar may not be a suitable approach to prevent *E. tenella* infection in chickens

Receptor1(R1)-Targeted Antigen Delivery Vaccines Enhance Activation of Chicken HD11 Cells *in vitro*

Oenone Bodman-Harris[1,2], Ellisha Kelly[2,3], Altaf Hussain[2], Jean-Remy Sadeyen[2], Wilhelm Gerner[2], Chrsitine Rollier[1], Munir Iqbal[2]

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Abstract

Macrophages are antigen-presenting cells (APCs) that play a central role in vaccine-induced immunity. To enhance antigen delivery to APCs, targeted antigen delivery vaccines (TADVs) have been developed using single-chain fragment variables (scFvs) specific for APC surface receptors fused to an antigen. Previous studies demonstrated that targeting the haemagglutinin antigen of H9N2 avian influenza virus (H9HA) to APCs enhanced immunogenicity, increased virus-neutralising antibodies, and reduced viral shedding in poultry. However, vaccine efficacy varied depending on the APC receptor targeted, with Receptor1(R1)-directed TADVs eliciting the strongest protection.

To investigate how scFv-specificity influences TADV potency, the chicken macrophage cell line HD11 was used *in vitro* to assess macrophage activation. HD11 cells were phenotypically characterised by confocal microscopy to confirm APC receptor expression. Macrophage activation was evaluated by

measuring nitric oxide (NO) production using the Griess assay following stimulation with viral antigens, scFvs, and TADVs.

H9HA, Newcastle disease virus fusion protein (NDV-F), antiR2 scFv, antiR3 scFv, and H9HA-antiR3 tested between 1.25 to 20µg/mL did not induce NO production above the assay detection limit. H9HA-antiR2 induced activation only at high concentrations but this response was lost by 1.25 µg/ml H9HA by molar ratio. In contrast, antiR1 scFv, H9HA-antiR1, and NDV-F-antiR1 induced strong and sustained HD11 activation, with antiR1 scFv able to activate HD11 cells at 0.078µg/mL.

These findings demonstrate that APC receptor targeting is an effective strategy for macrophage activation. Our data also suggest that scFvs targeting R1 may represent a broadly applicable adjuvant platform for enhancing poultry vaccination.

Chimeric fiber proteins of fowl adenoviruses (FAdVs) induce long-lasting humoral immune response in broiler breeders and their progeny

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Abstract

Fowl adenovirus (FAdV) vaccines based on recombinant fiber proteins have been shown to protect chickens from hepatitis-hydropericardium syndrome (HHS) and inclusion body hepatitis (IBH). Recently, recombinant chimeric fibers (crecFib-8b/8a and -4/11) combining epitopes from multiple serotypes were shown to provide simultaneous protection against HHS and the diverse aetiology of IBH. In the present study, the two constructs were combined in a single formulation to immunize commercial broiler breeders at 13- and 17-week-old to investigate their humoral response and the maternal antibodies (MatAbs) in progenies from eggs collected at 27-31- and 48-51-week-old.

Breeder hens presented pre-existing FAdV antibodies from field infection. However, after vaccination, ELISA-antibodies against both crecFibs remained at the highest measurable levels in immunized birds and their eggs until termination of the study (52-week-old), consistently higher than non-vaccinated control birds. Immunized birds also developed higher neutralizing antibodies (nAbs) against FAdV-8b and -11, whereas nAbs against FAdV-8a remained comparable to non-vaccinated birds. No FAdV-4 nAbs were recorded. Furthermore, consistently lower viral shedding was observed in vaccinated breeders.

The humoral response of vaccinated breeders' progenies was monitored until six weeks post hatching (wph), showing high levels until 2 wph, with crecFib-8b/8a antibodies waning slower than crecFib-4/11. NAbs against FAdV-8a and -8b were present up to 5 wph, with nAbs against FAdV-11 sporadically appearing in the progenies from older breeders.

These findings show that a vaccine formulation containing crecFib-8b/8a and -4/11 induces a strong and long-lasting humoral response in broiler breeders, with substantial MatAbs transfer to their progeny throughout the production period.

In ovo administration of *Clostridium perfringens* antigens elicits both humoral and cell-mediated immune responses in broiler chicks

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Abstract

Necrotic enteritis (NE), caused by *Clostridium perfringens*, is a major economic concern in antibiotic-free broiler production, yet effective *in ovo* vaccination strategies against NE remain largely unexplored. This study assessed the immunogenicity of three clostridial antigens delivered *in ovo* (18th day of incubation) in commercial broiler embryos. Upon hatching, chicks were housed by treatment group and euthanized at 2 and 3 weeks of age. The humoral and cell-mediated immune responses were evaluated by immunohistochemistry using anti-BU-1 (B cells) and anti-CD3 (T cells) antibodies in seven tissues: trachea, lung, duodenum, colorectum, cecal tonsils, spleen, and bursa of Fabricius.

All three antigens stimulated detectable immune responses compared to PBS-inoculated controls. One antigen consistently induced the most robust and widespread response, with significantly elevated CD3+ T cell percentages in trachea, lung, colorectum, cecal tonsils, spleen, and bursa of Fabricius at both time points ($p < 0.05$), as well as significantly higher BU-1+ B cell counts in trachea, duodenum, colorectum, and bursa of Fabricius. A second antigen elicited significant B and T cell responses primarily in the colorectum and bursa of Fabricius. The third antigen showed a more limited but notable response in the bursa of Fabricius at 3 weeks.

These findings demonstrate for the first time that clostridial antigens delivered *in ovo* can prime both mucosal and systemic adaptive immunity across multiple lymphoid and non-lymphoid tissues prior to the peak NE risk period, supporting the feasibility of an *in ovo* vaccination strategy against *C. perfringens* in broilers.

Protective and tissue-specific immune responses following *in ovo* Marek's disease virus mRNA vaccination

Janan Shoja Doost[1], Fatemeh Fazel[1], Ali Tolooe[1], Ayumi Matsuyama[1], Sarah K. Wootton[1], Shahriar Behboudi[2,3], Shayan Sharif[1]

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Abstract

Marek's disease virus (MDV) is a highly contagious oncogenic alphaherpesvirus threatening poultry production worldwide. Although conventional *in ovo* MDV vaccines are effective, incomplete protection and vaccine leakiness contribute to viral evolution. mRNA vaccines offer a flexible platform capable of inducing potent immune responses, yet their use against MDV remains largely unexplored *in ovo*. Here, we evaluated *in ovo* administration of an MDV mRNA vaccine encoding glycoprotein B (gB) and phosphoprotein 38 (pp38). Embryonated eggs were inoculated at embryonic day 18, and hatched chicks were challenged with very virulent RB1B at 5 days old. Vaccinated birds had significantly reduced lesion scores and tumour incidence compared with RB1B-infected controls, with tumour incidence reduced

from 100% in infected controls to 47% in vaccinated birds. Meq genome load in feather tips was reduced at 21 days post-infection (dpi), supporting decreased late viral burden. Organ weight indices showed lower spleen:bodyweight ratios in vaccinated birds relative to infected controls, while the bursa of Fabricius (BF):bodyweight ratio was lowest in vaccinated birds. Despite this BF outcome, protection against tumour development was maintained. Analysis of tissues using RT-PCR revealed organ- and time-dependent Meq expression in the spleen, BF, and lungs across 4, 10, and 21 dpi, indicating viral activity in target tissues. This occurred alongside tissue-specific alteration of IFN-α, IFN-β, and IFN-γ gene expression, suggesting vaccination shaped antiviral and cellular immune responses. Overall, *in ovo* MDV mRNA vaccination reduced pathological lesions while maintaining protection against tumour development despite tissue-level viral activity.

POSTERS

Poster presentations are divided across two dedicated sessions:

Even-numbered posters will be presented on **Wednesday** 2 September 2026, and **odd-numbered** posters will be presented on **Thursday** 3 September 2026.

Poster 1

Receptor-guided development of an avian reporter assay to distinguish chicken type I and type III interferons bioactivity

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Abstract

The Japanese quail CEC-32#511 Mx-luciferase reporter cell line is widely used to quantify chicken interferon (IFN) activity, but its responsiveness to type I versus type III IFNs has not been defined. Here, we delineated the receptor basis of this assay and generated a reporter cell line for selective quantification of chicken type III IFNs alongside the parental type I IFN reporter. Type I IFNs signal via IFNAR1/IFNAR2, whereas type III IFNs signal via IFNLR1 (IL-28Rα) together with IL-10Rβ. Comparative sequence analysis revealed high conservation of these receptor subunits between chicken and Japanese quail. However, RT-PCR with chicken- and quail-specific primers did not detect IFNLR1 (IL-28Rα) transcripts in parental CEC-32#511 cells under basal conditions or after influenza virus stimulation. Consistently, parental cells responded robustly to recombinant chicken IFN-α but not to recombinant chicken IFN-λ, demonstrating that the original reporter system specifically reflects type I IFN activity. To generate a type III IFN reporter, we stably expressed chicken IFNLR1 (IL-28Rα) in CEC-32#511 cells, restoring the limiting receptor chain required for IFN-λ sensing, and then knocked out endogenous quail IFNAR1 by CRISPR/Cas9 to abolish type I IFN responsiveness. Functional screening of selected single-cell-derived clones identified lines that completely lost responsiveness to recombinant IFN-α while responding to chicken IFN-λ in a concentration-dependent manner. These data define the specificity of the parental CEC-32#511 assay and establish validated paired avian reporter cell lines for standardized, class-specific quantification of chicken type I and type III IFN bioactivity in diverse biological and disease contexts.

Poster 2

Longitudinal analysis of vaccine-induced alterations in the faecal microbiota of chickens under conditions mimicking commercial rearing

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Abstract

The gut microbiota is crucial for immune development and overall health in chickens. In commercial production, birds routinely receive multiple vaccines during early life. While individual vaccines are

known to affect microbial composition, the impact of complex, multi-vaccine programs used in the industry is not well understood. This longitudinal study examined the impact of multiple live and inactivated vaccines, given at commercially relevant times from an early age, on gut microbial diversity and composition in layer chickens. We characterised microbiota profiles using 16S rRNA gene sequencing at pre- and post-vaccination timepoints across different vaccine groups. Overall, microbial diversity remained stable across most vaccines, indicating strong resilience of the gut microbiota to repeated immunological interventions. However, differential abundance analyses revealed vaccine-associated, temporal shifts in microbial composition by increasing aerobic or facultative genera and reducing short-chain fatty acid-producing taxa. Notably, these changes were not sustained, as the gut microbial community returned to a stable state after the vaccination schedule. These findings underscore the robustness of the chicken gut ecosystem and lay a foundation for future research into microbiome-vaccine interactions and their implications for poultry health, immunity, and production efficiency

Poster 3

B-cell heavy chain knockout chickens as a model for new insights into Infectious bursal disease pathogenesis.

Julia-Martha Hubrig[1], Johanna Trapp[1], Sonja Härtle[2], Benjamin Schusser[3], Silke Rautenschlein[1]

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Abstract

Infectious bursal disease virus (IBDV) is a significant immunosuppressive pathogen affecting chickens worldwide. Despite the wide variety of commercially available vaccines, vaccine-induced protection may still be insufficient. While it is well established that humoral immunity is important for the control of IBDV infection, open questions remain regarding the protective role of different T-cell subsets in the context of vaccination and infection.

We compared T-cell-mediated immune responses in B-cell-intact wild-type (WT) and B-cell heavy chain knockout ($JH^{-/-}$, KO) chickens following inoculation with an intermediate-plus IBDV (iIBDV) vaccine strain. Nineteen-day-old WT and KO chicken were inoculated with either a placebo (C) or iIBDV (I) and examined at 3, 7, and 21 days post-inoculation (dpi).

The two control groups (CWT, CKO) differed in the structure and size of the bursa of Fabricius. At 7 and 21 dpi, IWT birds displayed bursal atrophy, whereas no macroscopic differences were observed between IKO and CKO. Interestingly, IBDV-positive cells were detected by immunohistochemistry in the bursae of both infected groups. Both IWT and IKO chicken exhibited splenomegaly and, in some cases, mottled spleens at 3 and 7 dpi. While splenic lesions had largely resolved by 21 dpi in IWT birds, the spleens of IKO chickens remained enlarged compared with those of CKO.

Our study indicates that iIBDV infects birds even in the absence of functional B cells, resulting in more persistent splenic lesions than those observed in WT chickens. Possible differences in local immune responses between IKO and IWT chickens must be elucidated further.

Poster 4

Baicalin and baicalein differently modulate the innate immune response through NF-kB and JAK-STAT pathways in avian cell models

Matthéo Foulard[1,2], Emmanuel Kut[1], Vanaïque Guillory[1], Fabrice Robert[2], Delphine Gardan-Salmon[2], Sascha Trapp[1], Rodrigo Guabiraba[3]

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Abstract

Improving sustainability in poultry production while maintaining health and performance is a major challenge. Feed additives that enhance immune competence and resilience without impairing growth are attracting increasing interest. Flavonoids are promising candidates because of their antioxidant and immunoregulatory properties. Among them, baicalin and baicalein, two bioactive flavonoids isolated from the roots of *Scutellaria baicalensis*, have demonstrated anti-inflammatory and antioxidant activites in several animal species. However, their effects in avian cells remain poorly characterized. To investigate their impact on innate immune signaling, chicken lung epithelial cells and macrophages were exposed to baicalin or baicalein with viral-like stimuli. Cytotoxicity analyses revealed marked differences between the two compounds, with baicalein being more cytotoxic than baicalin, particularly in macrophages (15% vs. 95% cell viability after 6h at 30 µg/ml). These results were used to select non-cytotoxic concentrations for subsequent experiments. Cells were then co-stimulated for 6h with poly(I-C) and IFNα. In lung epithelial cells, baicalin reduced expression of CXCLi1 and the interferon-stimulated genes OASL and STAT1. STAT1 transcript levels decreased by approximately 40%f compared with IFNα treatment alone. In contrast, baicalein increased CXCLi1 and CXCLi2 expression while suppressing ISGs in a stimulus-dependent manner. In macrophages, reporter assays showed enhanced NF-kB activity following poly(I-C) co-treatment, with baicalein inducing the strongest activation (+150-fold relative to control). Western blot analyses demonstrated that both flavonoids reduced STAT1 phosphorylation and total STAT1 abundance. Together, these findings highlight distinct immunomodulatory effects through NF-kB and JAK-STAT pathways and support their potential as functional phytomolecules in poultry production.

Poster 6

Toward Testis-Mediated Gene Transfer in Birds: Evaluating Novel Delivery Approaches

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Abstract

Generating transgenic birds has traditionally relied on modifying primordial germ cells (PGCs) during embryonic development, a technically challenging and time-consuming process. In contrast, testis-mediated gene transfer (TMGT) has been successfully applied in several mammalian species, providing a simpler alternative for germline modification. However, similar strategies have not been developed for birds due to the lack of effective methods for delivering biological cargo into the avian testicular microenvironment.

Previous work from our laboratory demonstrated that adenoviral vectors successfully transduced Sertoli cells following intratesticular injection in chickens, although transduction decreased with age. This suggests that developmental changes in the testicular microenvironment, likely associated with maturation of the blood-testis barrier (BTB), reduce tissue accessibility.

Building on these results, we investigated additional delivery strategies that could help advance TMGT in birds. We administered purified GFP protein via intratesticular injection, with or without electroporation. We also injected GFP-labeled exosomes from HEK293 cells to assess their localization within the testis. These experiments were conducted in chicks at different post-hatch stages.

Preliminary results indicate that, from one to three weeks post-hatch, both GFP protein and exosomes reached the lumen of the seminiferous tubules following intratesticular injection. These findings suggest that, unlike in mammals, access to the seminiferous tubule lumen does not require delivery through the rete testis. However, neither electroporation nor exosome administration resulted in detectable intracellular uptake by seminiferous tubule cells.

Further studies are underway to optimize delivery into seminiferous tubule cells. These findings provide new insights into biological cargo delivery within the avian testicular microenvironment.

Poster 7

Blood leukocyte profiles correlate with *Ascaridia galli* larval and adult worm burdens

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Abstract

The parasitic nematode, *Ascaridia galli* represents an increasing problem for the poultry producers, especially in laying hens. Today, worm burdens in live birds are typically approximated using variables such as eggs per gram of excreta (EPG) in pooled samples. The aim of the current study was to evaluate correlations between larval or worm burden and leukocyte profiles in peripheral blood. Identification of robust correlates could e.g. allow longitudinal monitoring of single animals throughout infection, rather than relying on terminal endpoints, and improve the design of intervention studies. Moreover, peripheral blood parameters would provide information on systemic immune responses relating to events occurring at the site of infection.

Forty-eight Dekalb white chickens were infected with embryonated *A. galli* eggs at 1 week of age. At 2 weeks pi, 24 infected chickens were blood sampled and euthanised for larval enumeration, and at 7 weeks pi, a separate group of 24 infected chickens were blood sampled and euthanised for worm enumeration. Flow cytometric analyses were performed to phenotype and enumerate blood leukocyte subpopulations. The immune parameters were subsequently correlated to larvae and adult worm burdens. At week 2 pi, $CD4^+$ T cells were increased and CD4 expression negatively correlated with larval burden. The γδ T cells were increased at both weeks. TCR1 expression showed stage-dependent correlations, negative at week 2 and positive at week 7 pi, indicating dynamic modulation of T-cell phenotype during early invasion and later establishment of infection.

Poster 8

Deciphering *Salmonella* Gallinarum Δ*purB* effects on poultry PBMCs apoptosis, cell cycle and cellular proliferation

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Abstract

Salmonella enterica subspecie *enterica* serovar Gallinarum *causes* fowl typhoid and continues to threaten poultry health and productivity worldwide. The development of an attenuated strain provides a potential strategy for vaccine design; however, how these genetically modified strains influence host cellular responses remain vital. In this study we focused on a mutant strain that lacks the *purB* gene that is involved in purine biosynthesis and is essential for bacterial survival and growth. However, it's role in host immune cells has not been fully understood. This study aimed to decipher the effects of *purB* on host immune cell dynamics using an in vitro poultry peripheral blood mononuclear cells (PBMCs) model. PBMCs were infected with *Salmonella* Gallinarum wild type (*SG- WT) and Salmonella* Gallinarum Δ*purB* (*SG*- Δ*purB*) and flow cytometry analysis were performed to assess apoptosis, cell cycle and cellular proliferation. Results showed that the *SG*- WT induced 36% apoptosis and compared with 38% by the *SG*- Δ*purB* indicating no significant difference. Similarly, cell cycle analysis revealed no significant changes across the G0/ G1, S and G2/M phases. PBMCs proliferation increased increased progressively in uninfected control over time, whereas SG- WT and its ΔpurB mutant infection significantly inhibited proliferation at all-time points. Significant difference was found between uninfected and infected groups. These findings indicate that the deleted *purB* gene does not markedly influence PBMCs survival. Future studies may indicate longer incubation times and in vivo trials to validate these immunofindings.

Poster 9

Impact of vaccination on cytokine responses in turkeys after H9 LPAIV infection: Insights from tracheal and serum samples

Edit Kovács, Balázs Felföldi, Tímea Tatár-Kis, István Kiss

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Abstract

Low pathogenic avian influenza virus (LPAIV) subtype H9 is widely circulating in poultry, causing health concerns and economic losses in chickens and turkeys. This study evaluated the efficacy of a recombinant HVT-ND-H9 vaccine in commercial turkeys and assessed local and systemic cytokine responses following H9 LPAIV challenge.

Day-old turkeys (MDAND+, H9-) were vaccinated (Newflend ND H9, Ceva Animal Health) then challenged intranasally at 12 weeks of age with H9 LPAIV (G1 lineage/G5.5 clade). Samples collected at 0, 4, and 10-days-post-challenge (dpch) were analyzed for humoral immunity (HI test and ELISA) and viral load. IFN-γ and IL-10 concentrations in tracheal swab and serum samples were quantified using a magnetic bead-based multiplex immunoassay.

Vaccinated turkeys seroconverted before challenge, with antibody levels increasing thereafter. Although clinical signs were mild, substantial viral replication was detected in unvaccinated control birds, whereas vaccinated birds had significantly lower viral loads at 10 dpch.

At 4 days post-challenge (dpch), tracheal IFN-γ and IL-10 levels increased significantly, with more pronounced responses observed in unvaccinated birds. By 10-dpch, these differences had largely diminished, although elevated cytokine levels persisted in some individuals. Vaccinated birds displayed lower early cytokine responses following challenge, suggesting a more controlled local inflammatory reaction.

Systemically, serum IFN-γ concentrations increased by 10-dpch in both groups, with higher levels detected in unvaccinated birds. IL-10 responses followed a similar trend.

Overall, vaccination was associated with reduced viral loads and attenuated local and systemic cytokine responses, indicating enhanced control of H9 LPAIV infection and a reduced inflammatory response following challenge.

Poster 10

Oat-Dietary fibre changes phenotypic profiles of caecal intraepithelial lymphocytes (IEL) and cytokines during *Campylobacter jejuni* infection in broilers

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Abstract

The colonization of broilers by *Campylobacter jejuni* is a serious issue for meat safety. In the present study, we hypothesised that high dietary fibre (HDF) would limit colonisation without causing chronic inflammation. Broilers (n=40) were assigned to 4 groups (i) uninfected+basal diet, ii) infected+basal diet, iii) uninfected+oat-derived high dietary fibre (HDF) diet and iv) infected+HDF diet and inoculated at 14 days of age with *Campylbacter jejuni* or PBS respectively. Intraepithelial lymphocytes (IEL) cells were isolated at 14- and 21-days post infection (dpi) from the cecum and stained with antibodies ($CD45^+$, $CD3^+$, $CD4^+$, $CD8\alpha^+$, TCR-$\gamma\delta^+$, $Kul01^+$and $Bu1^+$ cells) to be quantified by flow cytometry. Additionally, gene expression of *TLR4, IFN-γ*, *IL-1β, IL-10* and *IL-13* was assessed by RT-qPCR in caecal tissue and loads of *C. jejuni* were determined. The HDF caused significant changes in the frequency of T cell subpopulations and gene expression of *IFN-γ* in uninfected chickens. At day 14 dpi, HDF-fed infected birds exhibited significant increases of $CD4^+$ T cells and B cells, alongside with reduced $CD8\alpha^+$, and TCR-γδ T cells compared to uninfected controls. These changes coincided with lower *TLR4, IFN-γ*, *IL-1β* and *IL-13* expressions. Beside this, decreased gene expression of *TLR4, IFN-γ, IL-1β and IL-13* in infected chickens fed with basal diet determined compared to uninfected chicken. By 21 dpi, HDF-fed infected birds showed less changes but increased $CD3^-CD8\alpha^+$ cells and 10.96-fold reduction in bacterial load. Overall, HDF induced modulation in the immune response against *C. jejuni*, accompanied with reduced bacterial colonization.

Poster 11

Deletion of the *Salmonella* Typhimurium *pcgL* gene enhances IFN-γ production in chicken TCRγδ$^+$ T cells

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Abstract

Salmonella enterica is a major pathogen of poultry and an important cause of foodborne disease worldwide. The *pcgL* gene encodes the D-Ala-D-Ala dipeptidase involved in peptidoglycan metabolism and has been associated with hypervirulence. However, its role in modulating avian host cellular immune responses remains unclear. This study investigated the influence of *pcgL* deletion on IFN-γ production by chicken T-cell subsets following *ex vivo* stimulation with *Salmonella* Typhimurium.

Splenic mononuclear cells were isolated from five naïve, adult specific pathogen-free (SPF) chickens and stimulated *ex vivo* with i) live or formalin-killed wild-type *Salmonella* Typhimurium 14028, ii) live or formalin-killed *pcgL* mutant, iii) live or formalin-killed complemented (*pcgL*$^+$) strain, iv) D-Ala-D-Ala, v) D-Ala-L-Ala, vi) PMA/ionomycin as stimulation control, or vii) unstimulated cells as a negative control. Following stimulation, intracellular cytokine staining was performed, and frequencies of IFN-γ-producing CD4$^+$, CD8α$^+$, and TCRγδ$^+$ lymphocytes were quantified by flow cytometry.

The *pcgL* mutant induced significantly higher frequencies of IFN-γ-producing TCRγδ$^+$ cells (mean 7.54%) than both the wild-type (mean 2.56%) and complemented (*pcgL*$^+$) strains (mean 2.44%). In contrast, CD4$^+$ and CD8α$^+$ T-cell subsets showed comparatively lower differences among the bacterial strains. D-Ala-D-Ala and D-Ala-L-Ala did not induce detectable IFN-γ production above unstimulated levels.

Hence, deletion of *pcgL* enhanced IFN-γ production by TCRγδ$^+$ cells. The findings suggest that alterations in *Salmonella enterica* peptidoglycan metabolism preferentially influence γδ T-cell activation, rather than extracellular contact with free D-Ala-containing dipeptides. This underlines the potential role of γδ T cells in the early avian immune response to *Salmonella* infection in chickens.

Poster 12

Deciphering Avian Immune Development: Insights from a Novel CRISPR/Cas9-mediated RAG1 Knockout Chicken Model

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Abstract

Birds are crucial models for immunological research, yet avian-specific immune development remains incompletely understood. We generated a novel immunodeficient chicken model using CRISPR/Cas9-

mediated Recombination Activating Gene 1 (*RAG1*) knockout chicken model to delineate classical and alternative immune developmental pathways. RAG1-deficient (*RAG1-/-*) embryos exhibited impaired B cell proliferation in the bursa of Fabricius due to disrupted B cell receptor signaling. Post-hatching, *RAG1-/-* chickens exhibited an absence of V(D)J recombination in peripheral blood mononuclear cells (PBMCs) and severe atrophy across lymphoid organs. Consequently, they lacked mature CD4+ and CD8+ T cells and showed drastically reduced serum immunoglobulins, confirming the essential dose-dependent role of RAG1 in classical adaptive immune development. Furthermore, scRNA-seq of the *RAG1-/-* spleen revealed two distinct Natural Killer (NK) cell subsets (NK-1 and NK-2). Despite divergent gene expression, these subsets share functional features—such as cytotoxicity and immune regulation—with human and murine NK cells. Strikingly, we identified a novel *RAG1*-independent atypical B cell (ABC) pathway. These ABCs, featuring attenuated ATR-mediated DNA damage responses, accumulated in the spleen and upregulated hyperinflammation-related genes, bypassing classical RAG1-dependence. In conclusion, the *RAG1-/-* chicken is a robust immunodeficient model that validates classical RAG1-dependent adaptive immunity while unveiling avian-specific alternative B cell ontogeny and conserved NK cell functionalities, offering profound insights into evolutionary immunology and biomedical applications.

Poster 13

Exploring factors driving susceptibility to highly pathogenic avian influenza across avian species

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Abstract

A new clade of highly pathogenic avian influenza (clade 2.3.4.4b HPAI) has spread globally since 2020, leading to a panzootic outbreak that has killed millions of wild birds and domestic poultry, causing significant ecological and economic damage. Compared to previously circulating viruses, clade 2.3.4.4b exhibits an expanded host range, with over 300 avian species newly infected since 2021. Here, we tested the ecological and host genetic correlates of interspecies variation in susceptibility to clade 2.3.4.4b HPAI. To this end, we extracted data from global reports on 2.3.4.4b infections for a subset of species found in the UK and conducted a systematic literature search to collect information on HPAI-associated morbidity and mortality. First, we tested whether host ecological traits (habitat, migratory status, gregariousness, and interspecies mixing) and life history (lifespan, adult survival, and clutch size) were associated with variation in susceptibility to HPAI. Second, we identified orthologues of genes known to be involved in the chicken and duck host responses to HPAI and investigated associations between sequence variation and selection in the coding and peptide sequences of each orthologue and susceptibility to HPAI. Identifying the ecological and genetic correlates of interspecific variation in susceptibility to HPAI is key to advancing our understanding of host-virus dynamics and identifying points of intervention to improve future HPAI management strategies.

Poster 14

The *rfaJ* gene deletion in *Salmonella* strains from serogroup D reduces intramacrophage bacterial survival

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Abstract

Salmonella Enteritidis (SE) is a major foodborne pathogen associated with the consumption of poultry products, while *Salmonella* Gallinarum (SG) provokes a high mortality, causing an important economic impact on the poultry industry. The O antigen does the primary immune host interaction, assisting with virulence factors and immune system evasion. Therefore, the absence of genes that codify this antigen production can be associated with recognition and survival of *Salmonella* spp. by macrophages. Our study aimed to evaluate the capture and survival of SE and SG, containing the knockout *rfaJ* gene, during infection of the chicken macrophage-like cell line HD-11. Bacterial strains *Salmonella* Enteritidis (str. P125109) and *Salmonella* Gallinarum biovar Gallinarum (str. 287/91) were used as background to *rfaJ* gene deletion through Lamda-Red technique. The experiment was conducted with a multiplicity of infection (MOI) 1:10 to evaluate uptake and survival at 2-, 4-, and 20-hours post-infection HD-11-line cells. Mann-Whitney test was used for uptake, while Two-way ANOVA with Šidák multiple comparisons test was used to evaluate the fold-net replication. The uptake had no difference, showing a similar recognition between the strains. However, on fold-net replication (2h vs uptake) and the survival rate, the mutants showed lower intra-macrophage replication, indicating bacterial clearance and reducing the survival rate when compared to wild-type. These findings suggest that the O antigen can play an important role in intracellular bacterial survival. The *rfaJ* gene can be explored as a potential way in immune response studies in *Salmonella* strains.

Poster 15

Chicken derived oviduct organ cultures as a model for investigation of innate immunity in the context of viral infection and reproductive status

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Abstract

Age and reproductive status may impact virus-host interaction in the context of various infectious pathogens, including Infectious bronchitis virus (IBV). IBV was shown to not only affect respiratory but also reproductive tissue in an age dependent manner. We may speculate that sexual maturity associated with hormonal status may impact host responses, as there is a close link between the hormonal network and cells of the immune system. Preliminary investigations indicated that oviduct organ cultures (OOC) derived from layer chickens at different age showed variations in the expression pattern not only of hormonal receptors but also of selected innate immune parameters. Further on, we compared OOC from three age groups (ag) (ag 1: 6-10 months; ag 2: 21–22 months, ag 3: moulting) for their susceptibility for IBV infection, and their subsequent antiviral innate immune response. The youngest age group showed the highest viral RNA loads, which correlated with lower background interleukin (IL)-6 and interferon (IFN)b mRNA expression levels in virus-free OOC from the same age group compared to ag3. As virus-infection of ag1 led different to ag3 to upregulation of these immune parameters, we may speculate that higher background expression levels of these innate parameters may provide a protective effect in the older age group, preventing high virus replication from the start. Interestingly, the background expression levels of IFN-λ and the upregulation rate after IBV inoculation

were comparable between age groups, suggesting no contribution of this antiviral factor to age variations in the IBV-innate immune response in OOC.

Poster 16

Novel Feather Follicle Stem Cell (FFSC) platform for generation of cell-free Marek's Disease Virus *in vitro*.

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Abstract

Feather Follicle is the only known anatomical site to date *in vivo* which facilitates the production of cell-free Marek's Disease Viruses (MDVs) shedding into the environment which can persist for months. Although MD vaccines provide protection against MD, they do not prevent viral infection and spread. Due to the highly cell-associated nature of MDV, the vaccines require extremely low temperature during storage and transportation to remain stable. To date, the factors that enable the production of cell-free MDV from the follicle have not been identified. In this project, we used Feather Follicle Stem Cells (FFSCs) isolated from the feather follicle to develop a novel platform for generation of cell-free MDV *in vitro* attempting to address the stability issue with the current cell-associated MDV vaccines. Through RT-qPCR and plaque assay, the kinetics of MDV replication were compared in chicken embryonic fibroblast (CEFs) and FFSCs across 3 serotypes; Rispens-CVI988; SB-1, and HVT. Through cycles of freeze-thawing and sonication, cell-free MDV lysate was obtained, and titre was quantified through standard plaque assay. We found that MDV replication is slower in FFSCs compared to CEFs suggesting involvement of unknown restricted factors. In addition, we also demonstrated that cell-free virus can be generated *in vitro* using FFSCs though the titre is varied. Further investigation into the factors involves in ability to generate cell-free MDV of FFSCs are still on-going. In conclusion, FFSCs not only have high potential for vaccine production aspect but also remain a valuable tool to understand MDV nature and host-viral interactions.

Poster 17

In vitro Bursal B cells maturation and susceptibility to Infectious bursal disease virus

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Abstract

Infectious bursal disease virus (IBDV) is the major immunosuppressive virus of young chickens, targeting and destroying bursal B cells. Here, we optimised culture conditions that support bursal B-cell viability and maturation and investigated how B-cell maturation influences susceptibility to IBDV infection.

Bursal B cells were cultured with CD40L alone, in combination with IL-4 or IL-21, or by sequential stimulation with CD40L + IL-4 followed by IL-21. To confirm functional maturation, bursal and splenic lymphocytes isolated from chickens vaccinated with an inactivated H5 vaccine were cultured under these conditions. Sequential cytokine stimulation significantly improved B-cell viability and promoted phenotypic maturation, characterised by a uniform population of large BU1-negative cell. Cells isolated from vaccinated birds cultured under the optimised conditions secreted Increased secretion of IgM and IgY, confirming functional maturation.

To investigate the effect of B-cell maturation on IBDV infection, bursal B cells isolated from naïve SPF chickens were infected with the classical, moderately pathogenic IBDV strain D78 or the highly attenuated strain PBG98 either immediately after isolation (day 0) or after 3 days of culture under the optimised maturation conditions. D78 induced greater viral replication, apoptosis, and expression of IL-1β, IL-6, CXCLi, TNF-α and IFN-γ than PBG98. Importantly, matured B cells exhibited significantly lower viral replication and reduced inflammatory cytokine responses than immature B cells following infection with either strain.

These findings identify B-cell maturation as a key determinant of susceptibility to IBDV infection and establish an improved in vitro model for investigating host–virus interactions in chicken B cells.

Poster 18

Chicken blood monocyte subtypes identified in response to *Erysipelothrix rhusiopathiae* infection

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Abstract

Blood monocyte subtypes have so far not been formally defined in chickens. Nonetheless, alterations in cell surface expression of the mannose receptor MRC1L-B and/or MHCII on chicken monocytes have been reported during bacterial infections. The present study aimed to evaluate if circulating monocyte phenotypes were altered in response to *Erysipelothrix rhusiopathiae* (ER) infection.

Blood samples from conventional laying hens in flocks with acute erysipelas outbreaks (3 flocks, n=62, n=36 bacteraemic) and healthy flocks (3 flocks, n=24) and experimentally ER infected SPF-chickens (n=26 infected, n=11 bacteraemic) and uninfected control chickens (n=25) were used. ER bacteraemia was assessed by blood culture. Flow cytometric analysis was performed using a no-lyse, no-wash immunolabelling protocol. Monocyte phenotype was determined with respect to expression of MRC1L-B and MHCII in all samples. An extended analysis including CD40, CD44 and CD45 expression was performed on laying hen samples.

In this cohort we identified four monocyte phenotypes; in hens from healthy flocks and in uninfected SPF-chickens MRC1L-B^{low}MHCII^{high} monocytes dominated. On day 1 after experimental ER infection MRC1L-B^{high}MHCII^{high} monocytes dominated regardless of subsequent bacteraemia while in SPF-chickens and in laying hens with ER bacteraemia MRC1L-B^{high}MHCII^{low} and/or MRC1L-B^{low}MHCII^{low} monocytes dominated. Expression of CD40, CD44 and CD45 also differed between the monocyte phenotypes.

We propose that MRC1L-B^{low}MHCII^{high} (CD40^{low}/CD44^{low}/CD45^{int}) constitute the “healthy”/steady state monocyte phenotype, MRC1L-B^{high}MHCII^{high} (CD40^{high}/CD44^{high}/CD45^{high}) the alerted and MRC1L-

$B^{high}MHCII^{low}$ ($CD40^{high}/CD44^{high}/CD45^{int}$) the highly activated monocyte phenotype, while the MRC1L-$B^{low}MHCII^{low}$ ($CD40^{low}/CD44^{low}/CD45^{low}$) monocyte phenotype may represent remission or exhaustion.

Poster 19

Towards understanding avian T cell development using a novel TCR knockout model

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Abstract

T cell receptor (TCR) knockout (KO) chickens provide a unique *in vivo* model to investigate the distinct roles of αβ and γδ T cells in avian immunity. Using CRISPR/Cas9-mediated genome editing, we generated TCR $C\beta^{-/-}$ and TCR $C\gamma^{-/-}$ chickens by deleting the constant region of the TCR β- or γ-chain, resulting in the loss of αβ or γδ T cells, respectively.

While γδ T cell-deficient chickens developed normally, TCR $C\beta^{-/-}$ chickens exhibited severe developmental and immunological defects, including granuloma formation, pronounced inflammatory responses, and impaired lymphoid organ development, making them unsuitable as models for T cell studies.

Similar immunological defects have been reported in TCR β-deficient mice, which display more severe phenotypes than TCR Cα knockout animals. This likely reflects thymic ontogeny: the TCR β-chain is expressed before the α-chain and promotes early thymocyte survival and differentiation. Consequently, disruption of the β-chain not only prevents mature TCR assembly but also perturbs thymic development.

To avoid the pathogenic phenotype seen in TCR β-deficient chickens, we aim to generate TCR $C\alpha^{-/-}$ chickens by targeting the constant region of the TCR α-chain using CRISPR/Cas9. This approach has been successfully established in DT40 cells, where efficient editing and precise integration were achieved. However, its application to primordial germ cells (PGCs) remains challenging. The successful generation of TCR α-chain knockout PGCs will enable the establishment of a viable TCR Cα knockout model to study αβ T cell biology while avoiding the severe developmental defects observed in TCR $C\beta^{-/-}$ birds.

Poster 20

Tylvalosin modulates IFN-γ production and immune gene expression in primary chicken immune cell

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Abstract

This study investigated the immunomodulatory effects of the macrolide tylvalosin (Aivlosin®; ECO Animal Health Ltd.) on chicken immune cells using an established ex vivo model. Mononuclear cells were isolated from the spleen and lungs of 13-week-old specific pathogen-free (SPF) layer chickens and divided into PMA/ionomycin-stimulated and unstimulated groups cultured with or without tylvalosin.

Intracellular IFN-γ production was assessed by flow cytometry in viable leukocyte subsets, including $CD45^+$, $CD4^+$, $CD8\alpha^+$ and TCR-$\gamma\delta^+$ cells. Additionally, RT-qPCR was performed to determine mRNA expression of Toll-like receptor genes (*TLR2B, TLR3, TLR4, TLR7*, and *TLR21*) and cytokines (*IFN-γ, IL-1β, IL-6,* and *IL-10*).

Flow cytometric analysis showed that tylvalosin significantly reduced IFN-γ production in PMA/ionomycin-stimulated $CD4^+$, $CD8\alpha^+$, and TCR-$\gamma\delta^+$ spleen-derived cells compared with stimulated cells cultured without the macrolide. A similar reduction was observed in stimulated lung-derived T-cell subsets but did not reach statistical significance. Gene expression analysis demonstrated that stimulation without tylvalosin significantly upregulated *IFN-γ* mRNA while downregulating *IL-1β* and *TLR2B, TLR3, TLR4, TLR7*, and *TLR21; IL-6* mRNA also decreased, although not significantly. Stimulation and tylvalosin addition induced similar expression effects, with *IL-6* significantly reduced in lung mononuclear cells, whereas *IL-1β* and *TLR4* exhibited a non-significant decline in spleen cells.

Overall, tylvalosin reduced IFN-γ-producing T-cell subsets after PMA/ionomycin stimulation, with significant changes in spleen-derived cells. Furthermore, the macrolide influenced immune regulation by altering cytokine and TLR expression at mRNA level. The findings suggest potential immunomodulatory effects of Aivlosin® on chicken mononuclear cells demonstrated by FCM and RT-qPCR.

Poster 21

Transcriptome response of γδ T cell deficient chickens after *Salmonella* Enteritidis infection

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Abstract

Salmonella remains a major cause of foodborne salmonellosis, with poultry serving as its primary reservoir. Although γδ T cells are abundant in chickens and localize to mucosal tissues, their contribution to intestinal immunity against *Salmonella* remains unclear.

Using TCR Cγ knockout (KO) and wild-type (WT) chickens orally infected with *Salmonella* Enteritidis, we previously demonstrated that KO animals exhibit increased bacterial dissemination with compensatory expansion of $CD8\alpha\alpha^+CD4^-$ αβ T cells. To determine how γδ T cell deficiency shapes intestinal transcriptional responses during infection, RNA-seq was performed on cecal wall and cecal tonsil samples collected 1-12 days post-infection (dpi).

Multidimensional scaling analysis identified distinct clustering by infection status, time point, and host genotype. Compared with controls, WT chickens mounted a transcriptional response peaking at 7 dpi, whereas KO chickens displayed a biphasic response at 5 and 9 dpi with substantially more differentially

expressed genes. Both genotypes activated core immune pathways, including cytokine production, Toll-like receptor signalling, and antimicrobial defence. Additionally, infected KO animals showed enrichment of pathways related to cell activation, receptor signalling, and inflammation. In cecum, KO chickens exhibited increased expression of *IL17A*, *IL17F*, *IL8* and oxidative stress-related genes compared with WT animals throughout infection. In cecal tonsils, infected KO chickens showed enhanced expression of interferon-stimulated genes, MHC class I-associated genes, and cytotoxic effector molecules.

These findings indicate that γδ T cell deficiency is associated with an enhanced inflammatory transcriptional response in the intestinal mucosa during *Salmonella* infection, likely reflecting both increased bacterial burden and altered immune regulation in KO animals.

Poster 22

Recombinant Interferon Gamma does not affect bacterial count in brown laying hens persistently infected with *Salmonella* Pullorum

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Abstract

Pullorum disease is caused by *Salmonella enterica* subsp. *enterica* serovar Gallinarum biovar Pullorum (SP), whose main transmission route is vertical, making its control necessary for the viability of artificial incubation and the development of poultry production, since animals that survive the disease become persistent carriers. Previous studies have suggested that SP infection promotes the induction of an M2 phenotype, in which macrophages exhibit an anti-inflammatory profile that favors the presence of bacteria within cells. Furthermore, the administration of recombinant Interferon Gamma (rIFN-γ) has already been associated with a greater macrophage activation and reduced survival of intracellular bacteria. Therefore, our study aimed to evaluate the effect of rIFN-γ administration on brown laying hens persistently infected with SP, when the bacteria are more efficiently able to manipulate the immune system. For the experiment, 0,2 mL of inoculum containing 9.5×10^8 CFU/mL of SP was administered at 6 days old. Then, 60 one-day-old brown laying hens were divided into three groups: group A was dosed with rIFN-γ at 5 days old; group B was dosed at 5 days old and, after 24h, received a boost; group C was the positive control. At 2, 4, 7, and 14 days post-infection, spleen and liver samples were collected for microbiological analysis. There was no significant difference between groups, neither in the spleen nor in the liver bacterial enumeration, potentially due to modulation of the inflammatory response. These results suggest the need for further analysis both in the assessment of tissue injury and gene expression.

Poster 23

Towards the Generation of CD1 and BG1 Knockout Chickens for the Investigation of γδ T Cell Functions and Ligand Recognition

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Abstract

Conventional αβ T cells are essential to adaptive immunity and have been extensively studied. In contrast, the functions of γδ T cells and the interaction with their respective ligands remain poorly understood. To better understand these cells, researchers are increasingly looking to chickens, which possess an unusually high abundance of γδ T cells, offering a unique model to study their role in the immune system. Although studies using wild-type and TCR γδ knockout chickens confirm that γδ T cells affect infection outcomes, the ligands involved are not well defined.

Among the potential ligands, we are mainly interested in CD1 and BG1 based on their distinct roles in avian immunity. CD1 molecules are conserved in chickens and present lipid antigens to γδ T cells, potentially facilitating the recognition of pathogens like *Salmonella*. BG1, by contrast, is a chicken-specific gene associated with resistance to Marek's disease and Rous sarcoma virus, though its functional mechanism remains largely unknown.

To define the functions of these potential ligands and to elucidate the mechanisms underlying γδ T cell activation, we are generating BG1 and CD1 knockout chickens using CRISPR/Cas9-mediated genome editing.

Poster 24

From controversy to clarity: B cell deficiency in chickens uncovers antibody mediated control of *Salmonella*

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Abstract

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 # These authors jointly supervised this work

Session: Adaptive Immunity

Salmonella enterica serovar Typhimurium is a major zoonotic pathogen in poultry, representing a significant public health risk through contaminated meat and eggs. The contribution of humoral immunity to *Salmonella* control remains controversial. This is because earlier studies relied on chemical or hormonal B cell depletion approaches that are often incomplete and may affect other leukocyte

populations or immune organs, complicating interpretation of B cell-specific phenotypes. To overcome these limitations, we utilised immunoglobulin heavy chain J segment knockout (JH-KO) chickens, which completely lack mature B cells while retaining an otherwise intact immune system. Wild-type and JH-KO birds were orally challenged with *S*. Typhimurium and monitored for clinical symptoms, survival, and bacterial colonisation of internal organs post-infection. JH-KO chickens exhibited markedly increased susceptibility compared to wild-type controls, characterised by higher systemic bacterial loads, more severe clinical signs, and elevated mortality. These findings suggest that humoral immunity plays a major role in the control of systemic Salmonella infection and demonstrate that JH-KO birds represent a powerful model to revisit long-standing controversies about the role of humoral immunity in avian salmonellosis.

Poster 25

Local responses to infection with the poultry roundworm *Ascaridia galli* assessed by RNAseq

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Abstract

Ascaridia galli causes significant problems in poultry husbandry, e.g. in free-range egg production. This nematode has a monoxenous life cycle including a "histotrophic phase" where larvae interact with and/or penetrate the intestinal mucosa. The present study aimed to increase knowledge on local host responses to early *A. galli* infection.

One week old B13-homozygous chickens were infected orally with 500 embryonated *A. galli* eggs/bird. Two weeks post infection (wpi), infected and age-matched uninfected chickens (n=6/group) were sacrificed, duodenal and jejunal tissues were collected and RNA was isolated. mRNA was sequenced, and differential gene expression was analysed.

The *A. galli* infection was confirmed as productive by faecal excretion of eggs (5 wpi) and recovery of adult worms (7 wpi) in parallel infected chickens. Gene expression at 2 wpi showed 35 duodenal and 225 jejunal differentially expressed genes (DEG). The majority of DEG in duodenum were also expressed in jejunum. The highest, ≥2 logFC, DEG were identified as involved in muscle contraction, mucus production and some immune related genes. Of the latter, *IL13* showed the highest expression and *IL9R, HRH4* and *LOC100858579* (granzyme G-like) showed prominent expression in both tissues. GO and KEGG analyses of DEG in jejunum were consistent with muscles and muscular contractions.

Thus, the local responses to *A. galli* infection were more prominent in jejunum compared to duodenum consistent with jejunum as a preferred site for larval invasion. Gene expression clearly indicated a host response of parasite expulsion, i.e. muscle contractions and mucus production, and a Th2-type response.

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