Local Th17 immunity upon mammary immunization is protective against E. coli mastitis

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ABSTRACT BOOK

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Results: Vaccine induced protection and individual calf IFN-γ responses to vaccination were variable, allowing us to investigate the correlation between observed protection at slaughter and immune response to vaccination and challenge. Load of MAP in tissues at termination was not associated with level of Vaccine-specific IFN-γ production at early or late time points after vaccination. Map-specific (PPDj) IFN-γ production appeared as a surrogate of disease with a direct positive correlation with level of MAP in tissues at slaughter. Polyfunctional T cells were induced by vaccination, but were not correlated with protection and could not be sustained during the long-term infection although Vaccine- and MAP-specific IFN-γ levels increased throughout the study period. **Conclusion:** Ag-specific IFN-γ response to vaccination is not a reliable predictor of protection and MAP-specific IFN-γ response to PPDj is a predictor of level of infection rather than level of immunocompetent response to challenge.

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Local Th17 immunity upon mammary immunization is protective against *E. coli* mastitis

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Current vaccines to *Escherichia coli* mastitis have shown some albeit limited efficacy. Their mode of action is poorly documented and the immune response protecting the mammary gland against *E. coli* is not completely understood. To improve our knowledge of mammary gland immune defenses, we compared the response of three groups of six cows that received either an intramuscular or an intramammary protocol of immunization against *E. coli* P4 before a homologous challenge. The control group received adjuvant only. Local immunization modified favorably the course of infection, by improving bacterial clearance while limiting inflammation. Systemic clinical score was also reduced and mammary secretion was preserved (Herry, 2017). High-throughput profiling using a newly developed cytokine 15-plex assay indicated a diminished TNFα production while increased IFNγ was detected in the immunized groups. Antibody response did not correlate with protection, but cellular immunity better related to protection of the mammary gland. Indeed, a transcriptome analysis performed using RNA sequencing on blood samples collected during immunization and infection phases shows that the lymphocyte response was activated in all groups 12 hours after inoculation and correlated with lower clinical scores as shown by weighted correlation network analysis. At the same time, neutrophils were produced and recruited with a moderate neutropenia in immunized groups. Type I interferon response correlated with intensity and persistence of inflammation during the late phase of mastitis. Furthermore, to assess the local T cell response in mammary tissues of locally immunized and control cows, CD4pos T cells were isolated by fluorescence cell sorting upon mammary tissue digestion of challenged and control glands, and their transcriptome determined by RNA sequencing. Results show that IL-17 expression was increased during *E. coli* infection and Th17 response was significantly enriched in immunized glands. These findings indicate

that protective mechanisms linked to local immunization rely on IL-17-mediated immunity.

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Type III interferon protects cattle against bovine viral diarrhea virus infection

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Background: Bovine viral diarrhea virus (BVDV) is an important pathogen of cattle that can cause significant economic losses in livestock industries, mainly due to reproduction failures and immunosuppression that predispose to opportunistic infections. It is known that infected animals elicit type-III interferon; however, there is no information on the antiviral activity of this cytokine in cattle.

Method: Recombinant bovine interferon lambda 3 (rIFNλ) was expressed in HEK-293 cells. Safety was assessed in 90-days-old calves (n=12) inoculated with two doses of 1.5, 3 and 6 U/kg, verifying the absence of hepatic and hematological toxicity. In a second experiment, 21 days-old BVDV-free calves (n=6) were infected with a BVDV-2 non-cytopathic field strain which produces a well-characterized respiratory disease. Calves (n=4) were inoculated with 6 U/kg of rINFλ two days before and after infection, while another group (n=2) was mock-treated. Presence of BVDV-NS3 protein and cytokines in serum samples and nasal swabs were measured using commercial ELISA kits from 2 to 21 days post-infection (dpi).

Results: Untreated calves developed respiratory disease, clinical signs appeared at 2 dpi and reached a maximum score at 4 dpi. Shedding was detected from 4 to 7 dpi and viremia persisted from 2 to 7 dpi. Systemic TNF-α was induced at 2 dpi. Conversely, rIFNλ-treated calves did not develop clinical disease, none of them shedded the virus and viremia was completely abolished in 3 of the calves, while the other animal had significantly lower NS3-serum levels ($p< 0.05$) than untreated-calves at all time-points. TNF-α was not detected and systemic IL-10 levels were increased**,** suggesting a role of rIFNλ in controlling inflammation.

Conclusion: These results demonstrate that rIFNλ is safe in cattle and capable of protecting these animals against the clinical disease caused by BVDV. This is the first report of the efficacy of type-III INF therapy in cattle.