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Abstracts

Keynote:

Novel vaccines against *T. gondii* in mice and ewes

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Primary infection of pregnant ewes with *Toxoplasma gondii* leads to abortion and significant economic losses for the livestock industry. Moreover, infected animals constitute the main parasitic reservoir for humans, since the meat is commonly eaten undercooked. Therefore, the development of a one-health vaccine seems the best prevention strategy. A vaccine constituted of total extract (TE) of *Toxoplasma gondii* proteins incorporated into biodegradable maltodextrin-based nanoparticles (DGNP) has been developed. This DGNP/TE platform induces specific Th1/Th17 cellular immune responses and vaccination led to 100% survival of mice with acute toxoplasmosis and to significant reduction in the number of brain cysts of mice with chronic toxoplasmosis or of offspring born to immunized mice infected during pregnancy (**1**). In ewes, immunization with DGNP/TE vaccine generated specific Th1-cellular immune response (**2**). No cerebral cyst could be detected in ewes after infection with *T. gondii* and vertical transmission of the parasite to lambs was drastically reduced (88%). Thus, DGNP/TE vaccine administered by the nasal route conferred a high level of protection against latent toxoplasmosis and its transplacental transmission in sheep, highlighting the potential for development of such a vaccine for studies in other species.

1. C. Ducournau, T.T. Nguyen, R. Carpentier, I. Lantier, S. Germon, F. Précausta, P.-J. Pisella, H. Leroux, N. Van Langendonck, D. Betbeder, I. Dimier-Poisson. Synthetic parasites: a successful mucosal nanoparticle vaccine against Toxoplasma congenital infection in mice, Future Microbiol. 2017; 12: 393–405. doi: 10.2217/fmb-2016-0146.
2. C. Ducournau, N. Moiré, R. Carpentier, P. Cantin, C. Herkt, I. Lantier, D. Betbeder, I Dimier-Poisson. Effective nanoparticle-based nasal vaccine against latent and congenital toxoplasmosis in sheep. Front. Immunol. 2020; 11:2183. doi: 10.3389/fimmu.2020.02183.

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Short presentation 1:

Discovering *Toxoplasma* genes involved in oocyst resistance and infectivity

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When a cat, the definitive host for *Toxoplasma gondii*, ingests tissues from an infected animal, the parasite invades the intestinal epithelium and ultimately form millions of oocysts that are shed within cat feces. Oocysts are highly stable, extremely resistant, and exceptionally infectious. However, despite playing a critical role in the transmission of *Toxoplasma*, oocysts, and the infectious sporozoites contained within, are under-studied. Therefore, our long-term goal is to **understand the genes and molecular factors involved in the resistance of oocysts and the invasion/replication of sporozoites.** We identified a number of candidate genes that could determine oocyst resistance or be essential for sporozoites, based on their specific expression in the late phase of sporozoite development. Among them, we selected a cluster of four putative late embryogenesis abundant domain-containing proteins (LEAs) and a PAN domain-containing protein (MIC4-like). We hypothesize that LEAs might be involved in the oocyst's extraordinary resistance, as these proteins have been shown to be involved in protecting plant seeds from desiccation, while MIC4-like might determine sporozoite invasion similar to what has been described for other PAN-domain-containing proteins. To investigate these hypotheses, we generated knockouts of the MIC4-like gene and the LEA cluster in the cat-compatible M4 strain. Our results show that these knockout parasites can form *in vitro* cysts and do not exhibit any growth defect compared to the wild-type M4 strain. Moreover, we were able to recover *in vivo* cysts of the MIC4-like knockout from mice and infect one cat orally. MIC4-like knockout oocysts collected from feces had no defects in sporulation, excystation or invasion. We hypothesized that, in the absence of the MIC4-like gene, MIC4, a homologue of MIC4-like, might be sufficient for sporozoite invasion. Thus, we generated a strain lacking both MIC4-like and MIC4, which, together with the LEA cluster knockout, will be assessed for oocyst resistance and sporozoite infectivity. If we succeed in finding oocyst and sporozoite essential genes, cats may be vaccinated with a strain unable to form infective oocysts, ultimately limiting the infection in humans and animals.

Short presentation 2:

Immunization in prepubertal cattle with *Neospora caninum* live tachyzoites failed to prevent fetal infection in pregnant cattle after experimental heterologous challenge

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The aim of the present study was to evaluate the safety and protective efficacy against vertical transmission in heifers inoculated with live tachyzoites from NC-Argentina LP1 strain at their puberty and challenged during pregnancy with a heterologous strain. Fifteen pregnant Angus heifers were involved in the present study: four animals inoculated subcutaneously (SC) with 1.106 live tachyzoites of NC-Argentina LP1 strain before reaching puberty (Hecker *et al*. 2019) and challenged intravenously (IV) with 1.108 live tachyzoites with NC-1 strain at 210 days of gestation (Group A); four animals received PBS SC before reaching puberty and were challenged IV with 1.108 live tachyzoites with NC-1 strain at 210 days of gestation (Group B), four animals inoculated SC with 1.106 live tachyzoites of NC-Argentina LP1 strain before reaching puberty (Hecker *et al*. 2019) and not challenged (Group C); and three animals have been received PBS SC before reaching puberty and were not challenged (Group D). Serum, placenta, peripheral blood mononuclear cells (PBMC) and umbilical cord samples were collected from dams and their calves at delivery before colostrum intake. The presence of antibodies in dams and calves was evaluated by indirect fluorescence antibody test, indirect ELISA and Immunoblot. Parasite DNA detection was assessed by nested PCR. Serological results showed that 4/4 dams and 3/4 newborn calves from Group A were seropositive to *N. caninum* at birth. In addition, one fetus from this group was aborted, but fetal fluid of this animal was seronegative to *N. caninum* and no parasitic DNA was detected in his tissues. In Group C, 4/4 dams and 1/4 newborn calves were seropositive to *N. caninum* at birth. All dams and calves from group B were *N. caninum*-seropositive and all dams and calves from group D remained *N. caninum*-seronegative. *N. caninum* DNA was amplified in two dams (placenta and PBMC) and one calf (umbilical cord) from Group A. In addition, *N. caninum* DNA was not detected in any placenta, PBMC or umbilical cord samples analyzed from Group B, C and D. The results of serology and PCR in heifers inoculated before puberty and challenge (Group A) showed that the specific immune response developed in the young animal (Hecker et al. 2019) was unable to prevent the congenital transmission after experimental

challenge with a heterologous strain. However, some degree of protective immune response against recrudesce of infection in dams inoculated before puberty could have been induced because although recrudescence happened, the congenital infection with NC-Argentina LP1 strain was evidenced by serology only in one calf in animals not challenge (Group C). Although anti-*N. caninum* antibodies were detected in dams and calves of group B and C, no *N. caninum* DNA was described in these groups. This fact could be associated to a low parasite load associated with a loss of virulence in the NC-1 strain, as has been described in previous works. Although the inoculation with live tachyzoites of NC-Argentina LP1 isolate in 6-month-old female calves generated specific cellular immune responses with specific antibody levels that decreased at day 120 PI (Hecker et al. 2019), it was unable to protect the fetuses against an heterologous challenge. Based on the findings of this study, our research group will continue characterizing the evolution of the immune response during pregnancy in dams. In addition, it would be deepening the studies in calves from Group A to determine if the infection in these animals was for the strain used as vaccine inoculum or strain used as challenge strain.

Short presentation 3:

Harnessing self-assembled peptide nanofiber to prime *robust* specific CD8 T cell response in mice

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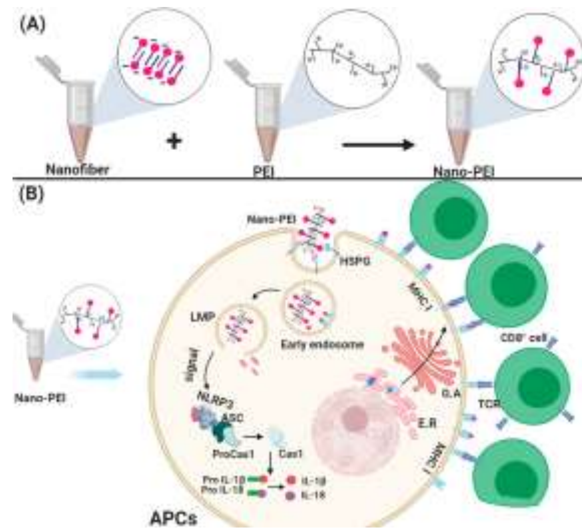

Induction of tumor-specific $CD8^+$ T cell response is known major challenge for cancer vaccine development. Here we optimized a vaccine delivery platform through formulation of epitope-bearing peptide nanofiber (Epi-Nano) with *polyethylene imine (PEI)* that can robustly prime specific $CD8^+$ T cell response. Nanostructural characterization revealed that formulation of peptide nanofibers with PEI (Epi-Nano-PEI) led to form short strand nanofibers with positive surface charge, reduce critical aggregating concentration (CAC), increase resistant to proteolytic degradation. In vitro and in vivo antigen uptake and cross presentation experiments demonstrated that Epi-Nano-PEI was significantly up taken more efficient with antigen presenting cells (APCs) in comparison to Epi-Nano, and was more potent than Epi-Nano to present exogenous epitope-MHC1 complex (cross presentation) in the PEI concentration-dependent manner. The finding can be due to induce lysosomal membrane permeabilization (LMP) by PEI. Moreover, Epi-Nano-PEI, in comparison to Epi-Nano, efficiently induced NLRP3 inflammasome pathway signaling, leading to the increased NLRP3 inflammasome-related cytokines (IL-1b and IL18) and IL-6. Viability study showed that Epi-Nano-PEI in contrast to PEI alone, interestingly not only did not possess cytotoxic effect even induced macrophage proliferation. Also, high number of antigen presenting cells (APCs) was detected in peritoneal space of mice intraperitoneally had received Epi-Nano-PEI compared those had received Epi-Nano or PBS. Finally, it demonstrated that Epi-Nano-PEI was robustly trigger specific $CD8^+$ T cell response rather than Epi-Nano with PEI up to 10 µg/mouse. Taken together, Epi-Nano-PEI is suggested as versatile promising vaccine platform to mount a robust specific $CD8^+$ T cell response against the target epitope.

Short presentation 4:

Development of a yeast-based vaccine system for oral delivery of *Eimeria tenella* antigens in commercial layer chickens

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Oral vaccines are relevant to the control of enteric diseases such as coccidiosis, caused by *Eimeria* parasites, as they can deliver specific antigens to the gastrointestinal tract and ideally stimulate an appropriate mucosal and systemic immune response. A number of antigens have been identified that are protective against *Eimeria tenella* challenge of chickens when delivered by recombinant protein or DNA vaccination; these include Apical Membrane Antigen 1 (AMA1), Immune Mapped Protein 1 (IMP1) and Microneme Protein 3 (MIC3). Now, a major challenge is

to find an effective method for cost-effective, flock-level delivery. The aim of this study was to use yeast to express and deliver recombinant protein for oral vaccination of chickens. Three antigens from *E. tenella* (Et) were selected for expression in *S. cerevisiae*: EtAMA1, EtIMP1 and EtMIC3. Antigen DNA sequences were initially cloned into a commercial plasmid (pYD1, Invitrogen) for expression on the surface of *S. cerevisiae*, confirmed by antibody staining and flow cytometry. Two *in vivo* vaccine challenge studies have been performed in layer chickens: a low-level challenge (250 oocysts per chicken) study examined EtAMA1 and EtIMP1, and a high dose (4,000 oocysts per chicken) challenge study which examined all three candidate antigens. In the low dose challenge study a significant decrease ($p<0.01$) in parasite replication was demonstrated using qPCR 5 days post-infection for Hy-Line Brown chickens vaccinated orally with *S. cerevisiae* expressing EtAMA1 (64.8%) or EtIMP1 (54.7%) alone, or combined (86.2%). In the high dose challenge study, a significant decrease in parasite replication was observed for Hy-Line Brown chickens vaccinated orally with *S. cerevisiae* expressing either EtAMA1 (60.6%) or EtIMP1 (66.0%) alone, for those given a mixture of *S. cerevisiae* with all three antigens together (45.9%)($p<0.01$). Average lesion scores were reduced, but not significantly lower in vaccinated chickens compared to the unvaccinated/challenged group. The use of a yeast-based system for delivering *E. tenella* antigens appears to be effective at reducing parasite replication in the caeca, although further optimisation is required for higher level challenges. If successful in larger trials, this system would provide a scalable non-GMO oral vaccination strategy with no requirement for a consistent cooling chain.