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THE INTERFERON GAMMA RESPONSE TO MYCOBACTERIUM AVIUM IN VITRO CAN BE CORRELATED WITH A HIGHER RISK OF CLINICAL KETOSIS IN DAIRY COWS

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Ketosis is a common metabolic disease often associated with reduction of immune competence and linked to other pathologies (mainly mastitis and metritis) of the transition dairy cow. These may be preceded by innate immune responses well before parturition [1,2]. The aim of this study was to evaluate the association between clinical ketosis after calving and adaptive immune responses during the transition period. Thirteen pluriparous Italian Friesian dairy cows were monitored from 21 days before till 28 days after calving. Cows were housed in a tie-stall barn with ad libitum feed and water. Diets were formulated to cover requirements according to the National Research Council Recommendations. During the trial, the animals' health status was checked every day and daily feed intake and rumination were recorded. After calving the cows were milked twice a day, and milk yield was recorded. Blood was collected from the jugular vein before the morning feeding, at different days from calving (DFC) from -21 till 28 DFC. Plasma samples were analyzed by a clinical analyzer (ILAB 650, Instrumentation Laboratory, USA) for energy parameters (glucose, NEFA and BHBA). Moreover, at -21 and 28 DFC a blood tube was collected and used in an IFN-gamma (IFNG) release assay for Mycobacterium avium on heparinized whole blood (internal method IZSLER, MP 13/011). Results were evaluated in terms of Delta OD (difference for IFNG between avian PPD-stimulated and control wells). Cows were retrospectively grouped according to their plasma BHBA concentrations after calving in Control (CTR, BHBA< 1.4 mmol/L; 7 cows) and Ketosis (KET, BHBA > 1.4 mmol/L; 6 cows). Data were analysed as a repeated measures study using the MIXED procedure of SAS with group (CTR or KET) as fixed effect. KET cows shower a lower feed intake during the 1st month of lactation with difference at 3 and 4 weeks after calving (P<0.05). Moreover, KET cows showed a lower milk production at 4th week (37.6 vs 40.3 kg/d of CTR, respectively; P<0.1) and, a lower rumination time (453 vs. 500 min/day of CTR; P<0.1). The markers of energetic metabolism confirmed the worse negative energy balance after calving in KET cows. In particular, BHBA concentrations were higher from -7 to 14 DFC (P<0.05), NEFA levels were higher from 0 to 28 DFC (P<0.05) and glucose concentrations were lower at 3 DFC (P<0.05). The IFNG response during challenge showed differences, both before (-21 DFC) and after calving (28 DFC), delta OD being higher in KET compared to CTR cows (P<0.05). In practice, cows mounting a higher IFNG response to Mycobacterium avium showed a higher risk to develop ketosis after calving. Considering that IFNG is related to metabolic pathways of energy use, a vigorous IFNG response to environmental microbial stressors may represent a risk after calving, when homeostatic control circuits are less effective. Our study confirms that peculiar features of the immune response of cows can affect the responses to metabolic changes after parturition.

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WATER BUFFALO SUBCLINICAL MASTITIS: CHANGES OF MILK MICROBIOTA AFTER TREATMENT WITH LACTOBACILLUS RHAMNOSUS

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Water buffaloes mastitis represents a major issue in terms of animal health, cost of therapy, premature culling and decreased milk yeld. The emergence of antibiotic resistance has led to investigating strategies in order to avoid or minimize the antibiotic use, especially during subclinical mastitis disease [1]. Use of bacteriophages, vaccines, nanoparticles, cytokines and also lactic acid bacteria may represent alternative strategies to antibiotics. Lactobacillus rhamnosus is part of the normal gut microflora, having meanwhile an immunostimulatory activity. The aim of this study was to investigate the change of milk microbiota after the theraputic treatment with Lactobacillus rhamnosus of mammary gland quarters affected by sub-clinical mastitis. A number of 64 quarters were included in the study, of which 43 affected by sub-clinical mastitis (no signs of clinical mastitis and aerobic culture positive for udder pathogens) and 21 quarters were healthy (with no clinical signs of mastitis during the present lactation, with two consecutive Somatic Cell Counts (SCC) values lower than 500,000 cells/ml and aerobic culture negative for udder pathogens (H)). The experimental design was as follows: of subclinical mastitis quarters, a total of 11 were treated with antibiotics (SC-AB), 15 with Lactobacillus rhamnosus (SC-LB) and 17 with PBS as control (SC-PBS). Of healthy quarters, a total of 11 were treated with Lactobacillus rhamnosus (H-LB), and 10 with PBS as negative control (H-PBS). Samples were collected at two time points, T0 and T5 (days) and V4 region of 16S rRNA gene was amplified by PCR and sequenced using Ion Torrent Personal Genome Machine. Raw data were analyzed using Quantitative Insight Into Microbial Ecology 2 software. As determined at T0, the healthy core milk microbiota, defined as the asset of microorganisms shared by all samples, was represented by Corynebacterium and Propionibacterium. On the contrary, no genera were shared between subclinical mastitis milk quarters samples. Alpha and beta diversity, which highlights differences within and among samples, showed statistically significance differences between healthy and subclinical mastitis samples at T0 (p=0.01 for Shannon index and p=0.007 for Weighted Unifrac Distance Matrix , respectively). The microbiota structure of subclinical mastitis quarters changed after antibiotic treatment (p=0.008 for Weighted Unifrac distance matrix). The abundance of Staphylococcus decreased from 40.8% in SC-AB-T0 to 3.4% in SC-AB-T5 group (p=0.008), while Methylobacterium increased from 0.9% to 6.5% % in SC-AB-T5 group (p=0.01). On the contrary, the treatment with Lactobacillus rhamnosus did not alter the microbiota structure of subclinical mastitis quarters; interestingly, at taxonomic level, Pseudomonas increased from 1.3% to 4% in T5 (p=0.02). Microbiota structure of healthy quarters did not change after Lactobacillus rhamnosus treatment: at taxonomic level, Lactobacillus and Sphyngomonas increased their relative abundance from T0 to T5 (p<0.05). In conclusion, healthy quarters treated with Lactobacillus rhamnosus showed no alteration in milk microbiota; however, in subclinical mastitis quarters, this bacteria did not seem to improve the original quarter status. More studies are needed to investigate the probiotic role in mastitis treatment.

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ROLE OF IGA ANTIBODIES IN PIG ORAL FLUIDS FOR THE CONTROL OF PRRS VIRUS INFECTION

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Introduction
The Porcine Reproductive and Respiratory Syndrome (PRRS) is a complex model of host/virus relationship, in which the role of adaptive immunity after infection is ill-defined [1]. More convincingly, clinical protection of pigs can be accounted for by (A) a reduced susceptibility of macrophages to PRRSV infection and replication; (B) an effective control over the inflammatory response caused by PRRSV and other environmental, infectious and non-infectious stressors. This kind of adaptation is likely to take place during the so-called "acclimatization" of gilts, i.e. the controlled exposure of PRRS-naïve gilts to PRRSV-infected pigs before the breeding period. In this respect, we had repeatedly observed on farm a clear correlation between a balanced IgA to IgG anti-PRRSV Ab ratio in oral fluids (OF) and block of environmental virus spread through OF samples of PRRSV-infected gilts. Vice versa, OF samples with peak IgG titers were correlated with peak PRRSV titers in OF, in agreement with major antibody-dependent enhancement (ADE) of virus replication (Amadori M., submitted).
Aim
Owing to the above, we decided to investigate the neutralization of PRRSV by OF samples with widely different IgA/IgG Ab titers to PRRSV in terms of s/p ratios in ELISA.
Materials and methods
IgA and IgG Ab to PRRSV in OF were measured by ELISA (kit IDEXX P3) with anti-Ig isotype conjugates. After overnight antibiotic treatment, OF samples were tested for yield reduction of different PRRSV strains in swine pulmonary alveolary macrophages and monocyte-derived macrophages. Yield reduction of PRRSV was measured by Real-time RT-PCR .
Results and conclusions
In agreement with our field studies, PRRSV yield reduction in pig macrophage cultures was shown to be critically dependent on the Ig antibody isotypes in OF samples, ADE being associated with IgG-rich samples. Most important, the extent of these effects varied as a function of the susceptibility to PRRSV replication of different macrophage batches, and also of the PRRSV strains under study. In particular, we could discriminate between ADE-positive and ADE-negative PRRSV strains. Next, we took to separating IgG and IgA in OF samples of PRRSV-infected pigs by means of protein A and size exclusion chromatography. The above results were confirmed by using separated Ig isotypes. In general, the combination of dimeric and monomeric IgA was correlated with the strongest reduction of PRRSV replication. Finally, we decided to check yield reduction in pig macrophages, pre-treated with separated Ig isotypes, washed, and then infected with different PRRSV strains. This treatment was also correlated with a substantial PRRSV yield reduction, which was Ab isotype and PRRSV strain-dependent, and went along with a down-regulation of CD163 and CD169 surface expression. On the whole, our data point at major role of IgA in the control of PRRSV excretion. This might be accomplished by extra or intracellular interaction of IgA Ab with PRRSV, as well as by signals leading to a reduced susceptibility of macrophages to PRRSV infection. Accordingly, major epigenetic regulations in pig macrophages exposed to PRRSV and/or OF antibodies should be investigated in the near future.
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CARACTERIZATION OF CF33 IN VITRO MODELS OF CANINE BREAST CANCER

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Mammary gland tumors (MGTs) are the most common neoplasms occurring in dogs; they are malignant in about 50% of the cases. Many studies on MGTs employed the CF33 cell line for in vitro tests; however, little is known about gene expression of parameters involved in the innate immune response, in DNA repair, in cell cycle regulation, in the ability to secrete cytokines, or in response to infectious or non-infectious stressor (1). The aim of our study was to evaluate the basal level of protein release and gene expression of pivotal molecules in the innate immune response and cell cycle regulation. We also investigated the ability of this cell line to respond to an infectious stressor. To this purpose, we performed two experiments; first, to evaluate the basal level of gene expression we selected a set of 31 immune-related and epithelial gene transcripts: the immune-related group were TNFA, IFNG, IFNb, IL1B, IL2, IL4, IL5, IL6, IL8, IL10, IL12, IL15, IL16, IL17, IL18, IL23, IL27, MYD88, NFKb/p65, TLR4, TLR5, MD2 and CD14, and the epithelial group were CD44, CXCR4, RAD51, p53, PTEN, Erb2, TGFB, BCRA. CF33 cells were grown until confluence at 37°C in DMEM enriched with 10% (v/v) fetal calf serum, a mixture of antibiotics and L-Glutamine 4 mM/L. Cells were tested at 37°, 39° and 42° passages; each experiment was repeated ten times. Next, we evaluated the ability of this cell line to respond to S. typhimurium and S. monofasica. Bacteria were sub-cultured for 2 h a 37°C and re-suspended at MOI 100 in DMEM and used to infect cells; untreated cells were employed as negative control. Bacterial penetration and innate immune response were evaluated as previously described (2). Differences between results were checked for significant differences by ANOVA. The significance threshold was set at $P< 0.05$. Total RNA extraction, RT-PCR and set-up of Real Time qPCR reactions were done as previously described (3). Ribosomal protein S5 was used as housekeeping gene. All the genes under study were expressed with the exception of IL1B, IL-2, IL10, IL15, IL17, IL-18, IL27, IFNG, p53 and CXCR4. In particular, TGFB was expressed in 12 out of 30 samples, IL4 in 16 of 30 wells, IL6 in 14 of 30 samples, IL12 in 4 out of 30, IL-23 in 10 out of 30 while TNFA was unexpressed in 20 out of 30 wells. Regarding TLRs, TLR4 was expressed in 2 out of 30 samples and TLR5 in 4 out of 30 wells. NFKb/p65 was expressed in 12 out of 30 wells, CD14 in 8 out of 30 and ErbB2 in 6 out of 30. The other genes under study were expressed in all samples. Concerning Salmonella treatment both strains caused up-regulation of IL-8 (P=0.0039) and CD14 (P=0.0060) while we observed down-regulation of MYD88 (P=0.0410), NFKb/p65 (P=0.0458), p53 (P=0.0120), MD2 (P=0.0310) and TLR5 (P=0.0022). Our results outline the basal expression in CF33 of important genes involved in innate immune response and the ability of this cell line to respond to an infectious stressor. Moreover, we observed the ability of different strains of Salmonella to cause an inflammatory response in this cancer cell line.
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MODULATION OF INNATE IMMUNITY IN KIDNEY EPITHELIAL CELLS BY INFECTIVE AND NON-INFECTIVE STRESSOR

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Keywords: cadmium, LPS, MDCK, innate immunity

Madin-Darby Canine Kidney (MDCK) are cell lines commonly used as models to study the characteristics of epithelial cells [1], they are involved to study viral infection of cells and in vaccine production [2]. Previous studies suggest the sensibility of MDCK to invasion and penetration of bacterial strain and the constitutive expression in MDCK of genes involved in the innate immunity response and cell cycle regulation [3]. However there are no data about the ability of these cell line to responde to infective and non infective stressor. The aim of this study was to evaluate the MDCK cells innate immunity response to non-infectious stressor (Cd2+) and infectious stressor (LPS). To this porpouse we performed two experiments; in the first step we evaluated the ability of MDCK to responde to Cadmium (Cd2+) a non-infectious stressor, that at molecular level induces a cellular stress Cells were treated with 20 µM of Cd2+ dissolved in DMEM culture medium. After 3 h and 24 h of treatment at 37°C in 5% CO_2, mRNA was extracted to test gene expression. The second experiment provided the treatment of cells with 1 µg/mL of lipopolysaccharide (LPS) from Escherichia coli O111:B4 recognized an infectious stressor. After 3 h and 24 h of incubation t 37°C in 5% CO_2 mRNA was extracted to study gene expression. In both experiments we tested the modulation of followed parameters involved in innate immune response: IL-8, IL-6, IL-1β, TLR1, TLR3, TLR5, TLR9, INOS, CD14, MYD88, P65, TLR4, MD2, IL-18. Experiments were carried out in quintuplicate; cells treated with medium only were used as negative control. Each test was repeated twice. After 3 h of Cd2+ treatment we observed up regulation ($P<0.05$) of important pro-inflammatory cytokines (IL-8, IL-6, IL1β), INOS, TLRs (TLR1, 9, 3, 5) and down regulation of CD14. Cd2+ treatment at 24 h determined up regulation of MYD88, p65, IL-18 and down regulation of INOS, IL-1β, MD2 and TLRs. After 3 h of LPS treatment we detected up regulation ($P<0.05$) of MYD88 and down regulation of INOS, CD14, IL1β, TLRs (TLR 5, 4). No significant differences were reported after 24 h of treatment. Our results show the ability of MDCK to responde to infectious and non-infectious stressor; in particular, Cd2+ and LPS to modulate innate immune response in terms of gene expression as a function of time. These data suggest a possible alteration of host-pathogen interactions due to inflammatory response and modulation of TLRs expression.

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YERSINIA ENTEROCOLITICA INTERACTION WITH JEJUNAL EPITHELIAL CELLS

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Yersinia enterocolitica are zoonotic bacteria able to infect humans and animals, recognized as the third cause of foodborne disease in Europe (EU) in terms of prevalence (1). Important studies highlighted the molecular basis of pathogenesis of Y. enterocolitica infection, while scanty data are available about other environmental 1A biotypes, often isolated in cases of foodborne disease but not included in pathogenicity studies. Owing to the above, the aim of our work was to verify the modulation of intestinal innate immunity by different Y. enterocolitica strains. In our study, overnight cultures of 5 different Y. enterocolitica strains: 1B (O:8, ail+, ystA+, inv+, myfA+, ymoA+); 1A (O:9, ystB+, inv+, ymoA+); 1A (O:5, ail+, ystB+, inv+, myfA+, ymoA+); 1A (O:8, ystB+, inv+, myfA+, ymoA+); 1A (O:5, ystA+, ystB+, inv+, myfA+, ymoA+) isolated from wild boar livers were sub-cultured for 1 h at 37 °C in BHI medium. Each bacterial strain was re-suspended at 100.000.000 CFU/ml in DMEM/F12 medium (2) and used to infect pig intestinal IPEC-J2 cells; untreated cells were employed as negative control. Innate immune responses were evaluated by real time PCR as previously described (2, 3). Differences between data sets were checked for significant differences by ANOVA, and the significance threshold was set at $P < 0.05$. Our results showed different abilities to modulate gene expression by the strains under study with respect to controls. In particular, Y. enterocolitica 1B determined a pro-inflammatory effect characterized by up-regulation of IL-8 ($P < 0.0001$) and TNF-α ($P=0.0024$), and decrease of antimicrobial peptide gene expression: bD3 ($P=0.049$), bD4 ($P=0.049$). At the same time we observed down-regulation of CD14 ($P=0.011$), MD2 ($P=0.004$), TLR1 ($P=0.0415$), TLR4 ($P=0.0038$) and TLR5($P=0.0360$). Y. enterocolitica 1A strain 1 caused a pro-inflammatory response with increased expression of IL-8 ($P=0.0002$), TNF-α ($P= 0.0045$), bD1 ($P=0.049$), bD2 ($P < 0.0001$) and down-regulation of NF-Kb1 ($P=0.0130$), bD4 ($P=0.0164$), MD2 ($P=0.0397$), and TLR4 ($P=0.0242$). Y. enterocolitica 1A strain 2 caused pro-inflammatory response with increased expression of IL-1β ($P=0.0168$), IL-8 (<0.0001), TNF-α ($P= 0.0076$), bD1 ($P=0.0010$), bD3 ($P=0.0086$) and down regulation of NF-Kb1 ($P=0.0392$), MYD88 ($P=0.0425$), MD2 ($P=0.0429$), TLR1 ($P=0.0256$) and TLR4 ($P=0.0212$). Y. enterocolitica 1A strain 3 determined a pro-inflammatory effect characterized by up-regulation of IL-1β ($P=0.0309$), IL-8 ($P=0.0493$), IL-18 ($P=0.0138$), and decrease of antimicrobial peptide gene expression: bD3 ($P=0.0010$), bD4 ($P=0.08t$). At the same time we observed down-regulation of CD14 ($P=0.011$), MD2 ($P=0.0132$), TLR1 ($P=0.0029$), TLR4 ($P=0.048$) and TLR5($P=0.0123$). Y. enterocolitica 1A strain 4 determined a pro-inflammatory effect characterized by up-regulation of IL-8 ($P < 0.0001$), TNF-α ($P=0.0071$), and decrease of antimicrobial peptide gene expression: bD1 ($P=0.0078$), bD3 ($P=0.0163$). The adopted cell line had been shown to give valuable information about pathogenicity of bacteria (4). Our data suggest a potential pathogenic role of 2 out of 4 Y. enterocolitica 1A strains under study and different interactions with the host.

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MASTITIS RESISTENCE IN HOLSTEIN AND IN RENDENA CATTLE BREEDS

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The selective pressure for increased milk production in Holstein Friesian dairy cows has led to their higher propensity to develop diseases in the transition period, including mastitis, when compared to less selected and lesser producing dairy breeds which are typically characterized by a higher resistance to diseases. With the aim of investigating the factors associated to this phenomenon, this study applied a multidisciplinary approach to compare innate immune response patterns, metabolic parameters, milk protein profiles and the milk microbiota in 6 Holstein and 4 Rendena cows reared in the same farm and under the same management conditions. Quarter milk samples and blood plasma were collected from all cows at dry-off (T1), and 1 (T2), 7-10 (T3) and 30 days after calving (T4). Quarter milk samples were subjected to bacteriological culture, characterization of the milk microbiota by 16S metagenomics, milk protein profiling by electrophoresis and densitometry, somatic cell counting, measurement of the inflammation marker cathelicidin and assessment of different innate immune-related mediators such as lysozyme, CD45, IL-1β, TNF-α, PTX3, IL-1R8. In parallel, the main inflammometabolic parameters were measured in blood plasma samples. Holstein cows showed a more severe fat mobilization and systemic inflammatory response at T2 and T3 in comparison with Rendena cows. Rendena cows showed a greater muscle mass (i.e. higher creatinine) and an increased amino acid mobilization immediately after calving compared to Holstein. Upon bacteriological analysis, contagious bacteria such as Staphylococcus aureus and Streptococcus agalactiae were absent, but significant differences were seen in the general composition of the milk microbiota of the two breeds. The taxonomic profiles of both breeds were dominated by Firmicutes (mostly Streptococcus (average HF=27.5%, REN=68.6%)), followed by Proteobacteria, Bacteriodetes, and Actinobacteria. However, Rendena cows showed a lower microbial diversity and a more stable microbiota along the transition period. Concerning the milk protein abundance profile, pronounced differences were seen in colostrum (T2), with significantly higher amounts of immunoglobulins and other immune-related proteins in Rendena. Adding to this, the expression of innate immune related genes such as PTX-3, IL-1β, TNF-α, as well as the CD45/KRT5 expression ratio in milk cells, indicating the epithelial and leukocyte components, respectively, was lower in Holstein Friesian compared with Rendena at T2. In conclusion, several differences were observed among breeds, in spite of the same farming conditions. The observations reported in this work present numerous hints on the factors that may provide autochthonous, more rustic breeds with a higher resistance to metabolic diseases and mastitis.

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POSTERS

BACTERIAL CONTAMINATIONS OF CELL CULTURES CAN BE DETECTED BY FLOW CYTOMETRY

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Introduction

Cell culture control for bacterial contaminations is mandatory to enable correct cell growth. Contaminated cell cultures are often subvital, showing growth delay and unreliable responses to immunological tests. Nowadays, sterility control is carried out by microbiological methods, mainly in thioglycolate and trypticase soy broth liquid media incubated at 30 - 37°C over three days, which is hardly enough to detect low-grade microbial growth or slow-growing bacteria, let alone bacteria with complex growth requirements.

Aim

Owing to the above, we decided to develop a method to detect bacterial contaminations in cell cultures on the basis of the scatter characteristics of E. coli.

Materials and methods

We used non-filtered and 0.22 micron-filtered cell culture samples. Samples were clarified and the supernatant was centrifuged at 10,000 g for 3 minutes; the pellet was resuspended in sterile saline and labeled with Baclight® live / dead kit containing Syto9 and Propidium iodide (PI) fluorochromes, to detect bacterial viability by flow cytometry [1] [2]. The assays were carried out in a Guava EasyCyte HT flow cytometer (Merck Millipore), using Incyte software.

Results and conclusions

By setting two gates i.e. R4 (low fluorescence) and R5 (high fluorescence) in a RED H-log x GRN H-log plot and analyzing a positive control containing only bacteria, we could discriminate between sterile and contaminated cell cultures. The morphological picture of a cell culture negative for bacterial contamination was characterized by events in R4 with limited presence in R5 and an upward oriented diagonal of events, the ratio R5/R4 being generally <1. Sample filtration profoundly alters the R5/R4 ratio which always drops to values by far <1. Also new events appear in R4, probably particles degraded as a result of the mechanical filtration stress, e.g. autofluorescent extracellular vesicles damaged after filtration, incorporating less fluorochrome and moving from R5 upper right to R4 lower left. On the other hand, in a contaminated sample ≥97% of events are concentrated in R5 as a cluster; in the contaminated, filtered sample the events cloud moves from R5 to R4 with only 1-2% of events remaining in R5. Experimental evidence indicates that contamination of cell culture flasks with bacteria in log phase growth diluted 1: 2000 can be detected just after two hours of incubation. In conclusion, early detection of bacterial contaminations in cell cultures is badly needed. In this respect, flow cytometry was shown to detect bacterial growth very early, well before the three day-period of a successful microbiological assay. The cytometric approach is substantially cost-effective, also on the basis of a large prevalence of false-negative samples in bacteriological assays.

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IL-1BETA: A POTENTIAL INDICATOR OF THE TOXICITY OF AUTOGENOUS VACCINES

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Interleukin-1ß (IL-1ß) is a pro-inflammatory cytokine mainly produced by activated macrophages and monocytes. The precursor (pro IL-1ß) is located at cytoplasmic level and must be cleaved by caspase-1 to generate the mature activated form [1]. Assessment of IL-1ß production by macrophages in response to vaccine antigens could help evaluate the safety and efficacy of the vaccine-induced immune response. Macrophages were obtained after differentiation of pig monocytes from peripheral blood mononuclear cells (PBMC) frozen in liquid nitrogen. PBMC were thawed quickly at 38°C and cultured at the concentration of 6 to 10 million cells/ml in RPMI 1640 medium. The suspension was seeded in 48-well plates and incubated at 37° to promote monocyte adhesion. After 2-3 h, a medium change was made with RPMI 1640 + 10% fetal calf serum (FCS) + Macrophage-Colony Stimulating Factor (M-CSF) at a concentration of 10 ng/ml to stimulate macrophage differentiation [2]. After 4 days of differentiation, the macrophages were incubated for 24 hours at 37 °C with different dilutions of bacterial vaccine antigens. In the tests, a negative and a positive control were included. The negative control consisted of RPMI 1640 + 10% Fetal Calf serum (FCS), only, and the positive one consisted of 50 µL/well of LPS O:111 B4 at 10 micrograms /mL in complete medium, followed by 17 µL/well of 150 mM ATP after 4 h at 37°C. At 24 h of incubation the cellular supernatant was collected and frozen at -80°C. Samples were analyzed for IL-1ß by "Duo set ELISA for Porcine IL-1ß/IL-1F2" (R&D System). Preliminary results reveal that each macrophage population shows different levels of basic activation profile, shown by the levels of IL-1ß in the negative control. Most important, the sensitivity of macrophages to vaccine antigens was shown to vary depending on their own basic activation. Non-activated cells respond effectively to the antigen, whereas activated cells display a substantial tolerance, that could also be linked to the culture period before the assay. In fact, macrophages and monocytes, that are exposed to endotoxin are rendered "tolerant" and manifest a profoundly altered response when rechallenged with bacterial endotoxin [3]. It is therefore crucial for standardization to start from a batch of low-activation cells, to standardize the period of cell differentiation and to choose a batch of suitable FCS, in order to have representative and replicable data. The proposed work aims to present the potential of this methodology in the field of autogenous vaccine efficacy and safety control. This kind of evaluation may also pave the way to new studies on the effectiveness of the immune response and the risk of toxicity of autogenous vaccines.

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CHARACTERIZATION OF THE INTERACTION OF DIVERSE VIRULENCE AFRICAN SWINE FEVER VIRUS STRAINS WITH MACROPHAGE SUBSETS

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African swine fever (ASF) is a devastating disease for which there is no vaccine available [1]. ASF virus (ASFV) has a tropism for cells of the myeloid lineage, including macrophages and dendritic cells (DC) [2]. Despite the central importance of macrophages for ASFV pathogenesis and the polarising effects of classical and alternative activation on macrophage phenotype/function, there are very few studies of the interaction of ASFV with activated macrophages. We therefore conducted an *in vitro* characterization of the interactions of porcine monocyte-derived unactivated (moM⦿), classically (moM1) and alternatively (moM2) activated monocyte-derived macrophages with ASFV strains of diverse virulence. Monocytes were differentiated using 50 ng of hM-CSF and were then left untreated or activated with IFN-⦿ and LPS (moM1) or IL-4 (moM2). Cells were infected with 1 multiplicity-of-infection (MOI) of a virulent (22653/14) or a low virulence (NH/P68) ASFV strains, along-side mock infected control. 21 hours post-infection (pi) the expression of ASFV proteins and surface markers were assessed with flow cytometry. At different time pi (3, 6, 9, 12, 21 hours) total RNA was extracted and retrotranscribed, then gene expression of IFN-⦿ and 17 different IFN-⦿ subtypes was determined by q-PCR. We observed that both isolates infected all the macrophage subsets, however NH/P68, but not 22653/14, down-regulated MHC class I and induced IFN-⦿ gene expression. These results revealed differences between ASFV strains, suggesting that virulent isolates are able to evade host immune response and promote their survival in infected pigs.

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IN VITRO IMMUNOLOGICAL PROPERTIES EXHIBITED BY DIFFERENT FRACTIONS EXTRACTED FROM THE MICROALGA CHLORELLA SOROKINIANA IN A SHEEP MODEL

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Marine microalgae have been proven to modulate immune system, lipid metabolism, gut function, and stress resistance [1]. Animal health conditions primarily depend on quality of the administrated feed [2]; as a consequence, feeding animals with feed enriched with extracts from natural substances, such as microalgae, could reinforce their immune and health status. Chlorella sorokinana (CS) is a unicellular microalga with the most suitable source of omega (ω)-3 and ω-6 PUFA; moreover, in the market of microalgae, CS is one the most diffuse together with Spirulina in relation to their high content of protein and nutritional value [3,4]. Chorella has been proposed as botanical food for its biological activities in order to rehinforce human health [5].The objective of the present experiment was to investigate the in vitro effects of the unsaponified fraction (UP), the acetylated unsaponified fraction (AUP), the total lipids fraction (TL) extracted and purified from Chlorella sorokiniana (CS) on proliferative response of sheep cells and on their cytokine profile. Peripheral blood mononuclear cells (PBMCs) from sheep blood were cultured at 37 °C for 24 h and treated with the UP fraction, the AUP fraction, and the TL fraction, extracted and purified from CS. Cells were activated with Concanavalin A (ConA, at final concentration of 5 µg/mL) and Lipopolysaccharide (LPS, at final concentration of 1 µg/mL). For each fraction, 0.0 mg/mL, 0.4 mg/mL and 0.8 mg/mL were tested on PBMCs. Negative Control was represented by wells with 100 µL of PBMC suspensions without mitogens. Positive Control was represented by wells containing 100 µL of PBMC suspensions treated with ConA and LPS. Cell-free supernatants from each well were collected until ELISA for the determination of IL-10, IL-1β and IL-6. Bromodeoxyuridine (BrdU) assay was performed on cells to measure cell proliferation. Extracts from CS affected sheep PBMC proliferation and cytokine production. A strong inhibitory action on proliferation was registered by UP at 0.4 mg/mL concentration showing the lowest proliferative response with respect to all the other extracts. Furthermore, UP extract at 0.8 mg/mL concentration was also characterized by an increased IL-10 production. Conversely, TL fraction at 0.4 mg/mL showed a cytokine profile characterized by increasing of IL-10, IL-6 and at a lesser extent of IL-1beta secretion. In conclusion, a biological effect of CS extracts in sheep model has been demonstrated, which makes the microalga extract eligible for a clinical trial aimed at reducing the overuse of antibiotics.

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