TERZO CONGRESSO NAZIONALE ISCCA

Stato: INVIATO - ID: 3503

Monoclonal Antibody Clone CC8 recognizes a polymorphic epitope of bovine CD4 antigen

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INTRODUCTION: The CD4 antigen has an important accessory function in the interaction between T lymphocytes and antigen-presenting cells (APC). The Bos taurus CD4 gene generates two transcript isoforms (X1 and X2) and 13 causative SNPs were identified in exons 2, 3 and 4 of both isoforms. Although the monoclonal antibody clone CC8 was widely used to recognize bovine CD4, no anomalous binding has been described in previous studies.

AIM OF STUDY: To investigate the lack of binding between CD4 antigen and monoclonal antibody clone CC8 in Pezzata Rossa Friulana cows.

MATERIALS AND METHODS: Fifty-four and thirty peripheral blood samples were collected in the peripartum period from 18 Pezzata Rossa Friulana (PR) and 10 Holstein (FR) dairy cows respectively. 50 µL of K_3EDTA or Heparin-anticoagulated whole blood were incubated for 15 min, at 4°C in the dark, with saturating concentrations of mAbs, in a two color flow cytometric assay: FITC/PE-conjugate (clone CC8), Alexa488-conjugate (clones: CACT138A, GC50A, ILA11a) CD4 mAbs and APC-conjugated CD3 mAb (clone MM1A). Quantum™ Simply Cellular® (Bangs Laboratories) microspheres were used to evaluate the antibody binding capacity (ABC) of surface CD4 using clone CC8. The samples, processed according to a stain-lyse-wash procedure, were acquired on Cytoflex flow cytometer analyzer (Beckman Coulter) and results analyzed with Kaluza v2.1 software (Beckman Coulter).

RESULTS: The clone CC8 labeled correctly only 8 PR (45%) and 5 (50%) FR animals. Unexpectedly, 3 PR (17%) cows showed a complete lack of antibody-binding to CD4 T lymphocytes. Interestingly, 7 PR (38%) cows, including two daughters of "mutated" animals (i.e. carrier animals of mutated allele) and 5 FR (50%) cows showed MFI and ABC values reduced by 50% compared to normal animals. Using CACT138A, GC50A and ILA11A clones, all animals exhibited always similar expression levels of CD4. No correlation was found between lack of binding and type of anticoagulant or conjugation, concentration, lot or producer of mAb.

CONCLUSIONS: Although these results should be confirmed by sequencing of CD4 gene, we can reasonably affirm: 1) the clone CC8 recognizes a polymorphic epitope of bovine CD4 antigen, 2) the mutated and normal alleles are codominant, 3) flow cytometry can be used to screen this mutated allele in wide populations of cattle.

Acknowledgments: Study supported by the research program "IMMA" (D.M.16850/7303/2016) funded by the Italian Ministry of Agriculture.