

## CHARACTERIZATION OF THE BLASTOGENIC RESPONSE TO LPS OF BOVINE PERIPHERAL BLOOD MONONUCLEAR CELLS

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Mitogens are diverse compounds of plant and microbial origin, widely employed to test immunocompetence. In healthy, non-immunocompromised hosts, they induce DNA synthesis and division of large leucocyte populations, which can be reasonably associated with the capacity to mount adaptive immune responses. The blastogenic response of bovine peripheral blood mononuclear cells (PBMC) to lipopolysaccharides (LPS) has been investigated for a long time in our laboratories. In particular, a possible correlation between blastogenic response to LPS and disease resistance of periparturient dairy cows had been observed in previous studies (Catalani et al., 2013): low responder cows presented a much higher frequency of disease cases after calving, compared with high responder animals. Owing to the above, different aspects of the blastogenic response to LPS were investigated on PBMC of healthy, dry (2) or lactating (26) Friesian cows, and the extent of the response was evaluated in a 72-hour BrDU incorporation assay, as previously described (Catalani et al., 2013). Unstimulated and BrDU-treated cells were used as negative control. Stimulation with LPS induced little if any increase of cell counts over 72 hours despite consistent, low to moderate BrDU incorporation in all the PBMC samples under study. Poor replication of LPS-stimulated PBMC was confirmed by cell cycle and cell growth flow cytometry analyses. In particular, LPS stimulation gave rise to very low percentages of S phase cells, sometimes lower than in control, unstimulated cells, as opposed to Concanavalin A-stimulated PBMC. Also, LPS-stimulated and BrDU-treated PBMC were submitted to magnetic separation using B and T cell-specific mAb, and Miltenyi anti-mouse IgG MicroBeads. Analysis of BrDU incorporation after stimulation with LPS showed that both B and CD4 T cells are involved in the blastogenic response to LPS, in contrast with current data based on human and murine models. Finally, as opposed to control cells, LPS-stimulated PBMC maintained the expression of IL-1beta and up-regulated both IDO and TDO2 genes (kynurenine pathway, endotoxin tolerance). Both control and LPS-stimulated PBMC down-regulated Ig light chain expression. On the whole, our data indicate that differences in the response to LPS could be accounted for by heterogeneity of responding cells (B and T lymphocytes), that could also cooperate with monocytes in induction and regulation of endotoxin tolerance.

Catalani, E., Amadori, M., Vitali, A., Lacetera, N., 2013. Short communication: Lymphoproliferative response to lipopolysaccharide and incidence of infections in periparturient dairy cows. *J. Dairy Sci.* 96, 7077-7081.