

A non-recoverable hybridoma limits the production of monoclonal antibodies against bovine trophoblast non-classical *NC3*00101* protein

Parveen Parasar^{1,†,*}, Amanda Wilhelm¹, William C Davis², Kenneth L White¹ & Christopher J Davies¹

¹Department of Animal, Dairy and Veterinary Sciences; School of Veterinary Medicine and Center for Integrated BioSystems, 4815 Old Main Hill, Utah State University, Logan, UT 84322-4815, USA

²Department of Veterinary Microbiology and Pathology, Bustad 329, Washington State University, Pullman, WA 99164-7040, USA

Received 02 September 2016; revised 14 July 2017

The non-classical MHC class I (*MHC-Ib*) proteins are important modulators of immune system during pregnancy favoring survival of the fetus. Contrary to ubiquitously expressed classical *MHC-I* proteins, *MHC-Ib* proteins are oligomeric, and expressed in specific cell/tissue types thus minimizing maternal immune-mediated rejection of fetal-allograft and a successful pregnancy. A unique characteristic of *MHC-Ib* glycoproteins is expression of surface and soluble isoforms due to alternative splicing phenomenon. Bovine fetal trophoblast cells, during the third trimester of pregnancy, express non-classical bovine leukocyte antigen class Ib (*BoLA-Ib*) antigens. *BoLA-NC3*00101*, is a non-classical class I allele from cattle AH11 haplotype and is expressed as cell surface isoform. However, lack of monoclonal antibody (mAb) hinders the development of specific assay to detect the soluble/secreted *BoLA-I* antigens released by fetal trophoblast cells. The objective of this study is to synthesize mAb specific to *NC3*00101* molecule to develop an isotype-matched ELISA to assess *BoLA-I* protein(s). We demonstrated that majority of *NC3*00101* hybridoma-supernatants were low-titer and showed inappreciable reactivity in ELISA and flow cytometry assays. We identified a clone of hybridoma (NC3-3*2) that secreted mAb specific to *BoLA-NC3*00101* as demonstrated with flow cytometry. NC3-3*2 clone secreted supernatant that captured *BoLA-NC3*00101* protein in ELISA. Unfortunately, despite several efforts we could not recover the cryopreserved hybridoma. Non-recoverability and instability, thus, limited production of *NC3*00101*-mAbs. This study provides the evidence that mice immunized with bovine class Ib proteins elicit a specific immunogenic response and warrants future studies into generation of stable hybridoma secreting antibodies against *BoLA-Ib* proteins using robust methods of fusion and cryopreservation of hybridomas.

Keywords: Bovine leukocyte antigen (*BoLA*), Non-classical *NC3*00101* protein, Monoclonal antibodies, Hybridoma, Trophoblast, P815 cell line