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Resumen de Trabajos Libres

Characterization of monoclonal antibodies against horse CD11c integrin by flow cytometry.

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Introduction. The horse, once hunted and later domesticated, helped advance human communication, transportation and health. Horses had a significant role in the transfer of language, culture, and technology, with a particular emphasis on antivenom research and development. Antivenoms used for treatment of envenomation caused by like snakes and scorpions, are mostly produced in horses (1).Since the first description of dendritic cells (DCs) by Ralph Steinman (2) , groundbreaking discoveries have emerged around this particular cell lineage, thanks to these technology and protocol establishment, nowadays is possible to describe DCs as potent inducers of immune responses, with the ability to stimulate other cells and also to induce immunological tolerance (3).The search for molecular markers and additional functions of DCs is quite active. The demonstration of DC subsets has extended the interest and importance of DC biology. The use of monoclonal antibodies in the species described has allowed to generally define DC subsets as lymphoid and myeloid. With the available information and reagents, it is possible to phenotypically describe horse DCs by their expression of CD4, CD11a, CD14, CD86, MHC-I, MHC-II, FLT3, CADM-1low, LFA-1, CD172a, CD44 (4,5,6,7) Here we describe the characterization by flow cytometry of the first murine monoclonal antibodies (mAb's) against horse CD11c. One of these mAb's, named mAb-1C4, is able to recognize intact cells with dendritic morphology in cryosections of horse lymph nodes and apparently does not cross-react with human cells in tonsil sections (8). Immune cell phenotype experiments were performed using MHC II (FITC), CD14 (AF700), and TLR4 (PE), one of the monoclonal antibodies (1C4) have been labeled with biotin and detected with streptavidin APC-Cy7. With this staining panel it was possible to detect $CD11c^{+}MHC\text{-}II^{+}TLR4^{+}CD14^{-}$ as a putative dendritic cell population.

Experimental Strategy

The PBMCs were obtained by density gradient centrifugation with Ficoll-Paque Plus (Amersham) following the manufacturer instructions. The PBMCs were incubated with the four different antibody clones (1C4, 4F6, 1H10 and 2D4) at three different concentration ($1x10^6$ g/L, $2x10^6$ g/L and $4x10^6$ g/L), with a secondary antibody α-mouse APC and analyzed on a BD Accuri™ C6 plus Flow cytometer. The 1C4 clone was selected as the best mAb and cells were labeled and incubated with other surface markers using the following antibodies: anti-horse MHCII-FITC, CD14-AF700, TLR4-PE and analyzed on a CytoFLEX flow cytometer (Beckman Coulter). An additional cross reactivity assay of the mAb ∝CD11c was performed and multiple alignment of the amino acid sequence of Domain I of the alpha subunit of integrin (CD11c) in horse, human, mouse, donkey.

Results.

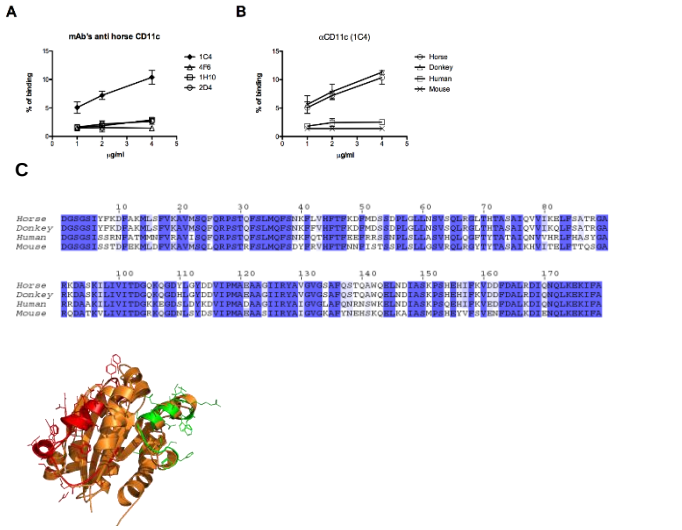

Fig. 1. Characterization of mAb 1C4. Titration of mAb αCD11c of horse, four mAbs were evaluated in their capacity of recognition in peripheral blood of three horses. Reconnaissance analysis of mAb 1C4 in peripheral blood cells of human, mouse, donkey and horse. In both experiments 50,000 events were obtained. Multiple alignment of the amino acid sequence of Domain I of the alpha subunit of integrin (CD11c), horse, human, mouse, donkey. Aligned and edited in Clustal omega and Jalview. Overlapping model of the three-dimensional structure of Domain I of the alpha subunit of the human and horse CD11c integrin, (model generated

by homology strategy in Phyrez). Dimensional structures were analyzed and edited with the Pymol program.

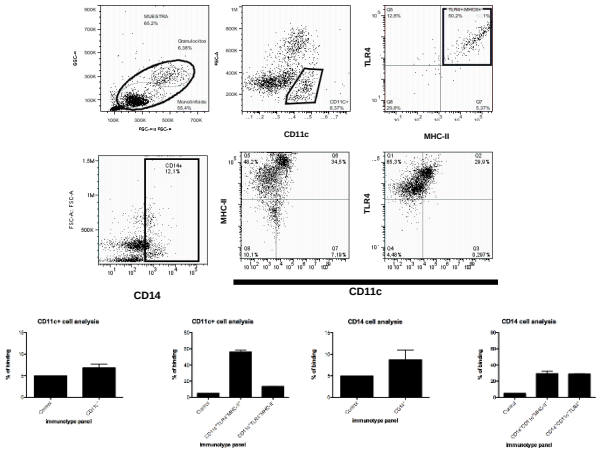

Fig. 2 Immunophenotyping of Horse CD11c + cells. Selection of positive CD11c population, coexpression of MHC-II and TLR4 was evaluated. Population CD14 +, co-expression of CD11c + -MHC-II + and CD11c + -TLR4 + was evaluated. Samples were analyzed by flow cytometry; 50,000 events were acquired. Representative figure of a horse.
C) Statistical analysis of comparison between the control and the populations. Dunnett method used.

Conclusions. Monoclonal antibody (mAb) 1C4 was selected according with the high affinity and recognition properties. A cross reactivity assay was performed in order to better understand if this mAb had the capabilities to recognize CD11c molecule in PBMCs obtained from human, mouse and donkey, in comparison with horse. Results obtained from this experiment shown highest percentages of binding, in equine and donkey samples, however, no binding was detected on human and mouse.
By doing a sequence and structural analysis of the Domain-I from donkey, horse, human and mouse it was identify two possible epitopes which can be recognized by the mAb 1C4, the differences of amino acid sequences can be related with the negative results obtained in human and mouse. On the other hand, it was possible to determinate a positive cell population of CD11c+ with positive expression for TLR4 and MHC-II in horse PBMC's. In spite, when CD14+ population was analyzed MHC-II+ CD11c+ and TLR4+ surface expression were detected.

Perspectives

- Multiple panel staining with other markers restrictive of DC.
- Cell stimulation with Ionomycin, LPS, PMA or ligands TLR to evaluate CD11c expression before stimulation.
- Characterization and immunophenotyping of equine monocytes with CD16/CD14, Stimulate and evaluate presence of CD11c
- Evaluate Donkey model

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