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Screening of anti-human leukocyte monoclonal antibodies for reactivity with equine leukocytes

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Abstract

Three hundred and seventy-nine monoclonal antibodies (mAbs) against various human CD molecules supplied to the HLDA8 animal homologues section (including four isotype controls) were analysed for cross-reactivity with equine leukocytes. First, flow cytometric identification of positively reacting mAbs was performed in one laboratory. Thereafter, a second round of flow cytometric evaluation was performed, involving three laboratories participating in the study.

The first test-round indicated 17 mAbs as potentially positive. After the second round of flow cytometric analysis, 14 mAbs remained (directed against CD2, CD11a, CD18, CD44, CD45, CD49d, CD91, CD163 and CD172) where cross-reactivity was anticipated based on similarities between the human and equine staining pattern.

Additionally, there was 1 mAb with weak likely positive reactivity, 12 mAbs with positive staining, which likely do not reflect valuable data, 5 mAbs with clear alternate expression pattern from that expected from humans, 5 mAbs with a questionable staining pattern itself, i.e. that was variable between the three labs, 32 mAbs with weak-positive expression and alternate staining pattern, and 279 negative mAbs (including the four isotype controls) were detected. In 31 cases, more appropriate target cells, such as thymocytes or stem cells, were not available for the screening. The results underline the value of this ''cross-reactivity'' approach for equine immunology. However, as only a few mAbs against leukocyte surface antigens reacted positively (approximately 4% of the mAbs submitted), the analysis of further anti-human mAbs and directed efforts to develop species-specific anti-CD mAb are still required.

Keywords: Horse; Immunology; Leukocyte differentiation

1. Introduction

Animal models have had a strong impact on immunology and the history of veterinary medicine is closely linked to horses. In immunology, horses played a major role ''producing'' the curative antibodies for Emil v. Behring. Since then, the military interest in horses declined and the immune response to

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disease in other (mostly livestock) animals received more attention. This was either due to their vulnerability to disease as food producing species (e.g. foot and mouth disease), carriers of zoonotic diseases (e.g. bovine spongiform encephalopathy), or models to study closely related human diseases (e.g. feline immunodeficiency). Large animals (swine) have also been used extensively to model xenotransplantation. As a result, the range of immunological tools available to study the immune response in cattle, sheep, pigs and chickens is acceptable. In contrast, especially equine and companion animal immunologists lack the diversity of tools, such as monoclonal antibodies (mAbs) directed against leukocytes, to investigate the immune response.

The immune system is an essential pillar of life and the genetic diversity of species is reflected therein. Although most immune mechanisms have been conserved, there is considerable diversity between the species due to the various life forms and challenges by pathogens requiring adaptation. The homology of equine versus human proteins of the immune system is 60–98% and the equine immune system is more closely related to humans than to the immune system of mice (Steinbach et al., 2002; Steinbach, unpublished observations). The homology of the proteins is not equally distributed over the amino acid sequences and there are often stretches of >15aa which are 100% conserved from humans to equine. Accordingly, there is a rational argument to test anti-human monoclonal antibodies for their cross-reactivity against equine immune cells. Additionally, a selection of anti-human mAbs, which are often available commercially, that cross-react with homologous proteins of other animal species have already provided valuable tools for veterinary medicine (e.g. Greenlee et al., 1987; Sopp and Howard, 1997; Pedersen et al., 2002; Paillot et al., 2005). However, caution must be exercised and cross-reactive monoclonal antibodies thoroughly characterised against the target species, since some reagents identify alternative epitopes on different proteins. For example B29A (VMRD, Pullman, Washington) recognises bovine CD5 but supposedly equine B lymphocytes (Kydd et al., 1994; Lunn et al., 1998).

Cell surface molecules are used for cellular communication with their environment including other cells and act as receptors for antigens, cytokines, antibodies, complement or as adhesion molecules. Many of them are expressed transiently and thereby reflect different stages of differentiation or activation. In the present study, flow cytometric analysis was used to screen mAbs supplied by various companies to the animal homologue section of the 8th international workshop on human leukocyte differentiation antigens (HLDA8) for reactivity on equine leukocytes. Taking into account some of the problems of previous workshops, we put strong emphasis on the comparability of equine and human staining patterns.

2. Material and methods

2.1. Isolation of leukocytes and mononuclear cells by gradient centrifugation

Fresh blood samples (~500 ml/sample) were collected from healthy horses of different breeds and sex into sterile blood bags with citrate solution (Baxter, Munich, Germany). Blood was slightly diluted with sterile PBS (40 ml blood + 10 ml PBS), aliquoted into sterile 50 ml tubes and centrifuged for 15 min at 1800 rpm (660 g), 20 °C (Kendro Multifuge 3S-R, BIOshield Rotor 7500 6435), to obtain buffy coats. After removing the upper plasma layer, the buffy coat area was collected into another centrifuge tube and an equal volume of room-temperature PBS was added.

Using 50 ml tubes again, the diluted buffy coat was layered on top of 16.5 ml Biocoll (Biochrom, Berlin, Germany, ρ 1.090) for separating leukocytes from erythrocytes. The gradient was centrifuged 20 min at 1600 rpm (520 g), 20 °C. Thereafter, the upper layer (plasma) was removed and the leukocyte fraction (including much of Biocoll solution) was collected carefully in order not to lose cells. Half of the leukocytes were diluted again with PBS and subjected to a second gradient centrifugation with Biocoll (ρ 1.077) for 20 min at 1600 rpm (520 g), 20 °C to isolate PBMC. These were collected and mixed with the leukocyte fraction resulting in a PBMC enriched pan-leukocyte preparation that was finally washed with PBS and centrifuged for 5 min at 1200 rpm (360 g), 20 °C. The cell pellet (which still included a lot of platelets) was resuspended in cold PBS to a final concentration of 1–2 $\times$ 10^6 cells/ml.

For analysis of CD markers known to be present in activated human cells only, equine leukocytes were isolated and separated as mentioned above. Activation of leukocytes, T cells, and B cells was performed using phorbol 12-myristate 13-acetate (PMA) at 20 ng/ml plus the Ca-Ionophor A23187 at 500 ng/ml for 12 h or phythaemagglutinin (PHA) at 5 μg/ml or LPS at 1 μg/ml for 2 days for lymphocytes (all reagents from Sigma, Germany). Additionally, after screening we also isolated monocytes via adherence, as described previously (Steinbach et al., 2005). From these monocytes, dendritic cells (DC) and macrophages (MΦ) were differentiated for screening of mAbs.