I. REVIEW OF LITERATURE

Animal models have had a strong impact on immunology and the history of veterinary medicine is closely linked to horses (*Equus caballus*). Horses are members of the order *Perissodactyla*, known as unpaired hoofed mammals, where families including rhino and tapir, are also found. Domestic and wild equids belong to the family *Equida*e, Genus *Equus* where six subgenera emerged (Groves, 2002): *Equus*, which contains the horses, and does not occur naturally in Africa; *Asinus*, which contains the true asses and is represented by a single species in Africa, the African wild ass (*Equus africanus*); *Hemionus*, which contains Asian wild asses; onager and kiangs; *Hippotigris*, which contains the mountain zebras; *Quagga*, which contains the Plains zebras; and *Dolichohippus*, which contains the Grevy's zebras in Africa. The equid specialist group of the IUCN recognised the African wild ass (*Equus africanus*) as a critically endangered species, the Grevy's zebra (*Equus grevyi*) and the mountain zebra (*Equus zebra*) as endangered (Moehlman, 2002). In immunology, domestic horses played a major role "producing" the curative antibodies for Emil v. Behring. Since then, the interest in horses declined and the immune response to disease in other livestock animals received more attention. This was mainly due to their vulnerability to disease as food producing species. As a result, equine immunologists lack the diversity of tools available for other livestock species, such as monoclonal antibodies (mAbs) directed against leukocytes and no mAbs tool-box is available for wild equids to investigate the immune response.

Monoclonal antibodies (mAbs) made the study of leukocytes surface antigens possible since they were developed by Köhler & Milstein (1975), enabling the production of an unlimited supply of a monospecific antibody. MAbs to leukocyte differentiation antigens allow for the identification and quantification of specific leukocyte sub-populations and enhance the comparative investigation of the role of effector cells in the control and pathogenesis of important diseases (Reinherz et al., 1979; Waldmann, 1989; Horejsi, 1991).

Changes in the state of a cell as it responds to signals delivered by soluble mediators as well as to the engagement of ligands displayed on cell surfaces or on extracellular matrices are the basis of differentiation and development. **Leukocyte surface glycoproteins** are distinct assortments of molecules which may be targeted by mAbs. Where a group of mAbs recognize epitopes on the same antigen, it will be clustered and assigned a "cluster designation" or CD number. CD antigens are part of the dynamic network of the immune system by which the immune system communicates within and among its major components such as T cells, B cells, NK cells, and myeloid cells. CD molecules play an important role in the immune

system, acting in antigen presentation (e.g. MHCI and MHCII molecules), as cytokine receptors (e.g. CD25), as signal transducing molecules (e.g. CD5), as receptors for complement (e.g. CD21), as adhesion molecules (e.g. CD11a/CD18), or play an important role in T cell function (e.g. CD28). As markers, leukocyte surface antigens can define subsets of leukocytes with restricted functional potential (e.g. CD14 on monocytes, CD4 on T_h cells and CD8 on T_c cells).

A large number of mAbs were produced recognizing leukocyte antigens in humans. To compare and classify these mAbs, a series of workshops was held to define their specificities against human leukocyte differentiation antigens (HLDA) by determining their expression on specific cell lineages and the characteristics of the antigens they recognize. The first **HLDA workshop** was held in Paris (1982). This initial meeting listed only fifteen agreed molecular entities, but it created an internationally agreed basis for the nomenclature of leukocyte molecules, and also provided a forum for reporting studies on their function and practical relevance (Mason et al., 2002). A further seven HLDA meetings have been held. The most recent was the 8th HLDA in Adelaide in December 2004 where 95 new CD designations were allocated and the list of designated human CD molecules expanded to 339 CD molecules (Zola et al., 2005).

Some laboratories around the world have produced and characterized mAbs to equine leukocytes, and a few workshops on equine leukocyte antigens were held to define the specificities of these mAbs to the antigens they recognize. At the **"First Equine Leukocyte Antigen Workshop" (ELAW I)** in Cambridge, England (1991) five laboratories participated and 86 mAbs against equine leukocyte antigens were analyzed. ELAW I established a standard procedure for naming equine leukocyte surface molecules when homologues to human counterparts as EqCD. Characterization of mAbs submitted to ELAW I based on determination of leukocyte expression pattern by flow cytometry, tissue distribution by immunohistochemistry (IHC), approximate molecular weights by immunoprecipitation (IP) and mAb functional assays in some instances. ELAW I confirmed the identification of several equine CD molecules described previously by individual laboratories. MAbs identified were directed against EqCD2 (WC3), clones HB88a and MAC288; EqCD4, clones CVS4 and HB61A; EqCD5, clones E23B, HB80A, HB19A, RVC1, HT23A and CVS5; EqCD8, clones HT14A, CVS8, RVC3 and HB20A; EqCD11a/CD18 (LFA-1), clones CZ3.2, CZ1.6, CVS9, H20A and CZ3.1; EqCD13 clone CVS19; EqCD44, clones BAG40A, BAT31A, H22A,

CVS18 and CZ5.15; EqMHCI, clones, CZ6 and MAC291 and EqMHCII, clones CZ11, EqT2 and CVS10 (Kydd et al., 1994).

"The second Equine Leukocyte Antigen Workshop" (ELAW II), held at 1995 in Davis, California, analyzed a panel of 113 mAbs from eight participating laboratories. ELAW II extended the characterization of 48 previously reported anti-leukocyte reagents (Kydd et al., 1994) and introduced 29 new anti-leukocyte reagents and 34 anti-equine immunoglobulin reagents. Analysis criteria were based on tissue distribution, biochemical characterization of the recognised antigen and functional data in some instances.

The clusters recognized by ELAW II in addition to those proposed in ELAW I are EqCD2, EqCD3, and EqCD28. In addition ELAW II assigned 12 new antibodies to these and established clusters, EqCD2, clone IFA6; EqCD3, clone F6G.3 (G12); EqCD5, clones F13C.3 (D9), EqT3 and clone TH2A10; EqCD8, clones CVS21, F18P.2, and F18H.2; EqCD44, clone CVS18; EqMHCI, clone CVS22; EqMHCII, clones F13A.3 and CVS20 (Lunn et al., 1998).

Clusters of antibodies identified by the ELAW I that were not apparently homologues to known human or mouse leukocyte molecules but shared characteristics were first assigned WC (workshop cluster) designations e.g. EqWC1, EqWC2, EqWC3 and EqWC4. ELAW II identified EqWC3 to detect EqCD2 and EqWC4 to detect EqCD28. Other groups of antibodies were established in both ELAW I and ELAW II to show exclusive reactivity with B lymphocytes, macrophages and granulocytes but an apparent homology or group could not be established. Despite the progress made at ELAW I and II, there are still substantial gaps in the armory of reagents available to study equine leukocyte biology and reagents able to characterize molecules of immunological importance like CD14, CD25, CD34, CD45 or CD206 were not available (Kydd et al., 1994; Lunn et al., 1998).

Leukocyte antigens which are of particular importance for this study shall be described in some more detail: **CD2 (LFA-2)** is a single chain type I transmembrane molecule consisting of 351 amino acids. This co-stimulatory molecule is a 50-60 kDa glycoprotein member of the Ig superfamily principally expressed by T and NK cells (Meuer et al., 1984; Moingeon et al., 1989). Low expression of CD2 has also been reported on subsets of thymic B cells, DC, Mo, and MΦ (Steinman, 1991; Shaw, 1995; Keogh et al., 1997). Crawford et al. (1999) suggested that $CD2^{+}$ $CD14^{high}$ Mo subpopulations consist of DC and detected CD2 on 10-18% of human monocytes by anti-human CD2 clone 39C1.5. CD2 is one of the cell adhesion molecules, and promotes the adherence of T-cells to target cells or antigen presenting cells (APC). Its ligand is CD58 (LFA-3), expressed on a wide variety of cells. Tavernor et al., (1994) cloned and

expressed equine CD2 cDNA. The predicted equine CD2 amino acid sequence had 50-65% identity with human, rat and mouse CD2 and greatest similarity shared with the human homologue. The molecular weight, T lymphocyte specificity and tissue distribution of the identified EqCD2 were most similar to the data described for human CD2. Three mAbs, HB88a, MAC288, and IFA6 were reported to detect the equine homologue of CD2 as they detected $EqCD5^+$ cells and recognised all cells in T-dependent regions of lymph nodes and the majority of mature medullary thymocytes together with many cortical thymocytes. In addition, all three mAbs recognized the transfected COS cells expressing equine CD2 in FACS analyses and mAb HB88a immunoprecipitated equine CD2 as a 58 kDa protein from T cells (Kydd et al., 1994; Tavernor et al., 1994; Tumas et al., 1994; Lunn et al., 1998).

The human **CD5** antigen is a 67 kDa pan-T lymphocyte marker with a probable role as an immunoregulatory molecule (Lydyard and Mackenzie, 1989), which may be involved in regulating responsiveness to Interleukin-1 (Nishimura et al., 1988a; Nishimura et al., 1988b). The natural ligand for CD5 is the B lymphocyte surface protein CD72 (DeFranco, 1991; Van de Velde et al., 1991). CD5 is also present, but at low density (approximately 20% of the density on T cells), on 10-25% of circulating and splenic human B cells, and the majority of B cells in human fetal spleen and cord blood (Kipps, 1989). Five monoclonal antibodies RVC1, HB80A, HB19A, HT23A, and CVS5 were assigned to the cluster recognizing EqCD5 in ELAW I, all of them stained equine T cells. HB80A, HB19A, HT23A, and CVS5 formed a particularly tight cluster and may bind to the same epitope, which resulted in blocking CVS5 binding in two-colour FACS analysis (Kydd et al., 1994). In ELAW II, anti-EqCD5 mAbs F13C.3 (Blanchard-Channel et al., 1994) and TH2A10 precipitated a molecule of approximately 69 kDa in reducing conditions and both of mAbs were T-cell restricted (Lunn et al., 1998).

Leukocyte integrins are a family of functionally important leukocytes adhesion molecules, composed of lymphocyte function-associated antigen-1 (LFA-1), Mac-1 and p150/95 proteins. These molecules are hetero-dimers which share a common β subunit (CD18) non-covalently associated with distinct α subunits which have approximate molecular weights of 180 kDa (CD11a), 170 kDa (CD11b) or 150 kDa (CD11c) (Kürzinger et al., 1981; Sanchez-Madrid et al., 1983; Hildreth and August, 1985; Springer et al., 1985).

The **CD11a/CD18 (LFA-1)** antigen is a member of the β2 integrin family, and functions as a cellular adhesion molecule (Kishimoto et al., 1987). EqCD11a/CD18 is comprised of a non-covalently linked heterodimer consisting of a 180 kDa α chain (CD11a), and a 100 kDa β

chain (CD18), expressed by all equine leukocytes and detected by mAbs CZ3.2, CZ1.6, CVS9, and H20A in ELAW I (Kydd et al., 1994; Lunn et al., 1998, Zhang et al., 1998). The mAb CZ3.1 failed to label lymphocytes of certain horses in two colour flow cytometry, but labelled granulocytes from all horses tested (Kydd et al., 1994; Zhang et al., 1998). Hogarth and McKenzie (1992) referred to this phenomenon in mouse to be due to polymorphism of the LFA-1 molecule. The mAb MHM23 directed against the human β subunit (CD18) cross-reacted with equine LFA-1 and immunoprecipitated both α and β subunits (Zhang et al., 1998).

CD11b is the 170 kDa integrin α chain expressed mainly on granulocytes, monocytes, and NK cells, as a non-covalently linked heterodimer with CD18. CD11b/CD18 (Mac-1), also known as the complement receptor type 3 (CR3), binds to the iC3b complement fragment on opsonised targets and mediates the subsequent ingestion process. Besides its importance for transendothelial migration of monocytes and neutrophils, Mac-1 is essential for Fc receptor-mediated neutrophil cytotoxicity and immunologic synapse formation (van Spriel et al., 2001; Hogg, 2002a).

CD11c is the 150 kDa integrin α chain highly expressed on monocytes, macrophages and NK cells. CD11c is expressed on granulocytes in moderate levels alike (Hogg, 2002b). p150/95 (CD11c/CD18) functions as an adhesion molecule binding to a receptor on stimulated endothelium (Stacker and Springer, 1991).

Human **CD14** is a 55 kDa glycosylphosphatidylinositol (GPI)-anchored membrane protein which functions as a receptor for the complex of lipopolysaccharide (LPS) and LPS-binding protein (LBP) i.e. a bacterial pattern recognition receptor (Pugin et al., 1993). CD14 binds LPS but is not capable of initiating a transmembrane activation signal since it does not contain a cytoplasmic domain. Upon LPS binding, CD14 physically associates with TLR4 which in turn transduces the signal (Aderem and Ulevitch, 2000). CD14 is primarily expressed on monocytes and most of tissue macrophages (Wright et al., 1990). Human granulocytes, particularly when activated, may express CD14 (Haziot et al., 1993; Rodeberg et al., 1997). Cells of the mononuclear-phagocyte series (MPS) are well defined by F4/80 in mice or CD14 in humans and other species (Hume and Gordon, 1983; Ziegler-Heitbrock and Ulevitch, 1993). While activating factors such as LPS up-regulate CD14, IL-4 leads to a decreased expression (Lauener et al., 1990; Marchant et al., 1992). Until recently, information on equine CD14 was scarce and although a number of anti-CD14 mAbs have been shown to cross-react with other species, attempts to find such a monoclonal for use in horses failed. The

cloning of equine CD14 (Genbank AF200416, Moore et al. unpublished) again argued for some cross-reactivities since the protein homology is 84% with 74% identical amino acids (aa).

CD21, the complement receptor type 2 (CR2), binds the complement fragments iC3b, C3dg and C3d, and interacts with CD23 (the low-affinity receptor for IgE). This 145 kDa glycoprotein plays a pivotal role in the activation and proliferation of B cells by lowering the signal threshold. While both purified B and T cell subpopulations express CD21 mRNA (Braun et al., 1998), only B cells, but not $CD4^+$ or $CD8^+$ T cells, express detectable amounts of CD21 on their cell surface. The number of antibodies for identifying equine B cells is small and the number that react with well-defined epitopes even smaller. Some human mAbs were analyzed for cross reactivity with horse CD21 by flow cytometry but no data about the molecular weight of the molecule detected. The monoclonal antibody, BU33, which is directed against human CD21, was shown to identify follicular lymphocytes in the lymph nodes and spleen of three horses, and a mean of 18 +/- 6% of peripheral blood lymphocytes from 10 horses by flow cytometry (Mayall et al., 2001). Human mAb B-Ly4 cross reacted with equine CD21 when analyzed by flow cytometry (Lin et al., 2002). Anti-human CD21 mAb BL13 was reported to recognise either different molecules or homologues that are expressed at different levels on horse cells (Merant et al., 2003).

The lymphocyte activation marker **CD25** is a 55 kDa glycoprotein, also known as the low-affinity Interleukin-2 receptor alpha chain (IL-2Rα). CD25 is expressed on activated T and B cells and macrophages. CD25 is also expressed by a 5-10% subset of non-activated $CD4^+$ T cells, some of which act as regulatory T cells (Sakaguchi et al., 1995; Dieckmann et al., 2001; Jonuleit et al., 2001; Sugamura, 2002). By itself, CD25 binds IL-2 only with low affinity. However, CD25 associates with CD122 (IL-2 receptor β chain) and CD132 (common cytokine receptor γ chain) to form the high affinity IL-2 receptor. The interaction of IL-2 with its cellular receptor triggers proliferation of the cells culminating in the emergence of effector T lymphocytes mediating helper, suppressor, or cytotoxic functions (Waldmann, 1986; Waldmann, 1991; Sugamura, 2002).

CD28 is an important co-stimulatory receptor in naïve T cells. *In vivo*, naïve T cells require two signals for full activation: an antigen-specific signal via the antigen receptor (TCR) and a second signal via a non-specific co-stimulatory receptor (CD28). TCR signals alone are insufficient to fully stimulate previously unstimulated (naïve) T lymphocytes to undergo clonal expansion and differentiation and these T cells can become anergic. Human CD28 is a

44 kDa homodimeric type I transmembrane glycoprotein heavily expressed on 95% of human $CD4^+$ cells, 50% of human $CD8^+$ cells, and plasma cells (Aruffo and Seed, 1987; Azuma et al., 1993; June et al., 1994). Two mAbs, HB68a and HB65a detected a small subset of $CD4^+$ equine T cells and immunoprecipitated a molecule of 46 kDa MW from surface iodinated PBMC. Immunoaffinity purification from equine thymocytes lysates using HB65A yielded two molecules of 46 kDa and 52 kDa when analyzed under reducing conditions and analysis under non-reducing conditions revealed the detection of a third, 92 kDa, molecule. The 52 kDa form was suggested to be the intracellular precursor to the 46 kDa mature protein since it was not immunoprecipitated from surface labelled PBMC and the 92 kDa band indicated a dimerization event of the detected molecule. Although the biochemical characteristics of the equine antigen detected by these mAbs are similar to that of CD28, the number of positive equine PBL differed considerably from human lymphocytes and lack of *in vitro* expressed equine CD28 made it difficult to designate the detected antigen, which was therefore clustered as EqWC4 in ELAW I (Kydd et al., 1994; Byrne et al., 1997).

Haematopoietic stem cells (HSC) possess the potential of self-renewal, proliferation, and differentiation toward different lineages of blood cells. These cells not only play a primordial role in haematopoietic development but also have important clinical applications (Mao et al., 1998). **CD34** is a surface glycophosphoprotein expressed on early lymphohematopoietic stem and progenitor cells (Katz et al., 1985; Andrews et al., 1986) and has been accepted as one of the most important molecules on human HSC (Civin et al., 1996; Burt, 1999; Handgretinger et al., 1999). Human CD34 is a heavily glycosylated type I transmembrane protein of approximately 116 kDa predicted molecular weight (Krause et al., 1993; Krause et al., 1996). Although the relative mobility of mouse CD34 varies slightly depending on the cell type in which it is expressed, the approximate molecular weight of mouse CD34 is 110 kDa (Krause et al., 1994; Krause et al., 1996). Despite the importance of CD34 as a marker of early haematopoietic stem/progenitor cells in experimental and clinical haematopoiesis, the function of CD34 has remained elusive. CD34 is involved in adhesion and localisation "homing" of haematopoietic cells to bone marrow stroma (May et al., 1994; Springer, 1994) and has a role in preventing the terminal differentiation of myeloid cells, and thus maintaining the cells at an immature haematopoietic stage (Fackler et al., 1992). Clinical applications of CD34 mAbs and purified $CD34^+$ haematopoietic cells in human are numerous and rapidly expanding. CD34 is also used as a marker for leukaemia diagnosis and subclassification, as a

label for quantification of stem/progenitor cells in blood and marrow, and as a tool for immunologic purification of stem/progenitor cells (Greaves et al., 1995).

CD44, a glycosylated transmembrane protein of 65-100 kDa, is widely expressed on the surface of most cell types (Stoll et al., 1989). CD44 mediates adhesion of leukocytes to endothelial cells, is involved in T lymphocytes activation (Horejsi, 1991), leukocyte attachment to and rolling on endothelial cells, homing to peripheral lymphoid organs and to sites of inflammation (Miyake et al., 1990). Leukocyte aggregation and signaling through CD44 induces cytokine release and T cell activation. The "classical" CD44 in humans consists of exons 1-5, 15-17 and 19, but a variety of alternate CD44 variants (CD44v) exist with variable inclusion of exons 6-14 (Lynch, 2004). Five mAbs; BAG40A, BAT31A, H22A, CVS18 and CZ5.15, were clustered at the ELAW I to recognise equine CD44. All of these mAbs recognised a protein of the EqCD44 molecule expressed in COS cells (Tavernor et al., 1993; Kydd et al., 1994). In flow cytometry analysis, this cluster of mAbs generally labelled all T lymphocytes, but only about 35-70% of the B cells appeared to be positive suggesting that these mAbs label B lymphocytes with a low intensity. Only CVS18 immunoprecipitated equine CD44 as a smear of approximately 100 kDa indicating heavy glycosylation of equine CD44 (Kydd et al., 1994).

CD45 (formerly termed B220, T200, or LCA=leukocyte common antigen) is a tyrosine phosphatase receptor expressed on all nucleated haemopoietic cells. The phosphatase activity of CD45 is essential for T- and B-lymphocyte antigen receptor signal transduction by regulating the activity of Src family tyrosine kinases such as $p56^{lck}$ (Alexander, 2000; Penninger et al., 2001; Hermiston et al., 2003). For CD45 cassette exons occur, which are variably expressed on haematopoietic cells (for review Hermiston et al., 2003; Lynch, 2004). The three variable exons are also termed A (4), B (5), and C (6) and the existing 5 isoforms in humans (in other species there may be more than 8) may be distinguished by mAbs against CD45RA, the 220 kDa isoform (detecting all isoforms with exon 4), CD45RB, the 200 kDa isoform (isoforms with exon 5) and RO, the 180 kDa isoform (isoform without exons 4-6) (Janeway, 1992; Lynch, 2004). The pattern of CD45 isoform expression changes during differentiation and activation of leukocytes and some cells express more than one isoform of CD45. In humans, lymphocytes expressing CD45RA are naïve T cells which, when activated, switch to expressing the smaller CD45RO molecule as the memory phenotype (Akbar et al., 1988; Clement et al., 1988; Serra et al., 1988). $CD45RA^{+}RO^{+}$ has been identified as the phenotype of cells undergoing a transition from naïve to memory status because of the early

expression of CD45RO isoform and the relative delay in the loss of CD45RA from the cell surface during stimulation (Deans et al., 1989; Salmon et al., 1989). In humans, there is a reverse CD45RB expression on CD45RA and CD45RO cells (Salmon et al., 1994). Human $CD45RA^+$ T cells express high levels of CD45RB, whereas $CD45RO^+$ cells express little CD45RB. In mice, the level of expression of CD45RB on $CD4^+$ T cells is believed to distinguish naïve ($CD45RB^{high}$) from activated/memory ($CD45RB^{low}$) cells (Powrie et al., 1996). Additionally, the binding of some mAbs to CD45 is influenced by the glycosylation pattern (reviewed in Trowbridge and Thomas, 1994).

The **CD49** gene family members (CD49a-f) are also known as the α1-α6 integrins that non-covalently associate with CD29 (β1-integrin) to form the very late antigen 1-6 (VLA 1-6) hetero-dimer. The expression of the CD49 genes differs significantly between the various leukocyte subpopulations. CD49d is a member of the integrin α chain family of 150 kDa approximate molecular weight, has a broad range of cellular expression including all leukocytes except neutrophils (Tanaka, 2002). Functions of CD49d are adhesion to cell surface ligands VCAM-1 and MAdCAM-1, contributing to lymphocyte migration from circulation into tissues e.g. homing of T cell subsets to Peyer's patches by the binding of α4β7 to MAdCAM-1 (Tanaka, 2002).

CD68 (macrosialin or gp110) is a 110 kDa transmembrane (type I) glycoprotein expressed intracellularly in cytoplasmic granules of monocytes, macrophages, and dendritic cells (Stockinger, 1989; Goyert, 2002b). CD68 belongs to a family of acidic, highly glycosylated lysosomal-associated membrane proteins (lamps) that include lamp-1 (CD107a) and lamp-2 (CD107b). While the function of CD68 is unknown, lamps are the major component of lysosomal membranes and may protect the membranes from attack by acid hydrolases. It is not clear whether the surface expression of CD68 is functionally significant or due to leakage from the lysosomes, as could be the case for the lamps. Inflammatory agents upregulate macrosialin expression.

CD83 is a member of the immunoglobulin supergene family with an approximate molecular weight of 43 kDa. CD83 expression in humans is slightly complex. In blood, only a population of DC stains positive. The marker has therefore been suggested also as the unique marker for mature DC. However, CD83 was originally cloned from activated B cells and was also detected in LCs, which are certainly the hallmark cells for immature myeloid DC (Zhou et al., 1992; Kozlow et al., 1993). Additionally, CD83 may at least on MoDC be induced during maturation and accordingly may appear on myeloid cells (Zhou and Tedder, 1995).

CD91 is part of an α/β hetero-dimer of the LDL receptor family binding to α_2-macroglobulin. CD91 β subunit is approximately 85 kDa in molecular weight containing one transmembrane segment and a short cytoplasmic region signaling for endocytosis. The extracellular region binds non-covalently to the 515 kDa α-subunit. The only haematopoietic cells that express CD91 are cells of the monocytic lineage and erythroblasts (Moestrup, 2002).

CD163, also previously termed M130 or RM3/1 antigen, is a 130 kDa integral membrane protein of monocytes/macrophages and a member of the scavenger receptor cysteine-rich family (SRCR) (Hogger et al., 1998). Previous studies with Mac 2-48, RM3/1, Ber-Mac3, and other monoclonal antibodies indicated that different values up to 100% of $CD14^+$ human monocytes were positive for CD163, as detected by flow cytometry (Backe et al., 1991; Hogger, 1998; Sulahian et al., 2000; Philippidis et al., 2004). CD163 is a candidate for diagnostic use as a marker of monocyte/macrophage activity in inflammatory diseases (Aristoteli et al., 2006; Baeten et al., 2004; Moller et al., 2004; Philippidis et al., 2004). CD163 has at least three alternate splice-variants two of which modify the cytoplasmic domain and do not alter antibody binding. The only extracellular alternative variant results in a 33 aa insertion between two SRCR-domains and most likely does not affect antibody binding either (Ritter et al., 1999). The swine homologue of CD163 is the 2A10 antigen (Sanchez et al., 1999). It has been shown that CD163 has at least two distinct functions: the clearance of hemoglobin and participation in anti-inflammation, here exhibiting cytokine-like functions as a soluble factor (Moller, et al., 2002; Moller, et al., 2003).

Signal-regulatory proteins (SIRP) are glycosylated, Ig-like cell surface receptors expressed on myeloid and neural cells. Human **CD172a** (SIRPα protein), approximately 110 kDa, inhibits cell signaling by receptor tyrosine kinases and cytokine receptors by recruiting phosphatases to the membrane via inhibition motifs. The extra cellular part of rat SIRPα contains 15 potential N-glycosylation sites and a large number of serines and threonines, which might become O-glycosylated (Fujioka et al., 1996; Sano et al., 1997; Adams et al., 1998; Saginario et al., 1998). The difference in molecular mass observed between myeloid SIRPα (110 kDa) and neuronal SIRPα (85-90 kDa) suggest that SIRPα is tissue-specific glycosylated (Sano et al., 1989; Sano et al., 1990; Veillette et al., 1998; van den Nieuwenhof et al., 2001). Unlike SIRPα, SIRPβ (CD172b) lacks the ability to recruit SH2 domain-containing protein phosphatases. However, SIRPβ associates with the transmembrane protein DAP12/KARAP, and stimulation of this complex induces tyrosine phosphorylation, MAPK activation, and

cellular activation. Therefore SIRPα and SIRPβ may play complementary roles in signal regulation (Kharitonenkov et al., 1997; Timms et al., 1998; Seiffert et al., 1999; Seiffert et al., 2001; Simmons and Vernon-Wilson, 2002). MAbs against the homologous pig protein were previously grouped as the SWC-3 (Lunney et al., 1994). SIRP-1α (CD172a) is the variant with two ITIM-motifs at the cytoplasmatic tail. SIRP-1α binds to CD47, whereas SIRP-1β is assumed not to bind CD47 (Seiffert et al., 2001). The binding of CD47 and SIRP-1α modulates macrophage/DC activity and has been proposed to deliver an inhibitory signal (Oldenborg et al., 2001). In this context it has been suggested that CD47 is a kind of "self" signal that controls macrophage activity via CD172a (van den Berg et al., 2004).

CD206 is the macrophage mannose receptor (MMR) also termed mannosyl-fucosyl-receptor (MFR). CD206, approximately a 162 kDa type I membrane protein (Ramkumar et al., 2002), is an important receptor for pinocytosis and phagocytosis that recognizes appropriately configured carbohydrate ligands on target molecules. CD206 is absent on monocytes but up-regulated during differentiation to macrophages (Stahl and Gordon, 1982; Ezekowitz et al., 1990). The upregulation of CD206 during *in vitro* culture is well known in humans (Shepherd et al., 1982). CD206 thereby distinguishes between PBMo and activated monocytes/macrophages. Additionally, CD206 is expressed on immature but not on mature DC (Sallusto and Lanzavecchia, 1994).

The toll like receptor 3 (TLR 3) was recently classified **CD283** (Zola et al., 2005). This receptor which detects dsRNA is located mainly intracellular (Sen and Sarkar, 2005). TLR3 recognizes viral dsRNA and its synthetic analogue polyinosinic-polycytidylic acid (poly I:C). TLR3 expression is commonly considered to be restricted to dendritic cells, NK cells, and fibroblasts but also activated human γδ and αβ T lymphocytes express TLR3 and signaling of TLR3 is differentially regulated in TCR-stimulated γδ and αβ T cells, suggesting an early activation of γδ T cells in antiviral immunity (Wesch et al., 2006). TLR3 signals lead to the production of IFN-β and causes dendritic cells to mature (Yamamoto et al., 2002).

The equine **Major Histocompatibility Complex (MHC)** genes are located on chromosome 20 (Antczak, 1989). Both EqMHCI and EqMHCII genes have been cloned (Barbis et al., 1994b; Szalai et al., 1994a, b). At least two polymorphic class I loci have been identified: ELA-A and ELA-B (Bernoco et al., 1987a; Lazary et al., 1988; Antczak, 1989). Monoclonal antibodies specifically produced against equine MHC class I (Donaldson et al., 1988; Kydd et al., 1991, 1994; Lunn et al., 1998) and MHC class II have been described (Crepaldi et al.,

1986; Monos et al., 1989; Lunn et al., 1998; Kydd et al., 1994). Due to the extensive conservation of the structure of MHC antigens between species, mAbs against MHC antigens of other species were found to cross-react with horse MHC (Crepaldi et al, 1986; Monos et al., 1989).

EqMHC class I molecules encoded by equine major histocompatibility complex (MHC) class I genes are composed of a 44 kDa transmembrane heavy chain and a 14 kDa non-covalently linked light chain termed beta 2 microglobulin (β_2M) (Antczak, 1992; Kydd et al., 1994). Class I MHC molecules are highly polymorphic, expressed on the vast majority of cells within the body, and are critical to the presentation of peptides to lymphocytes that express the CD8. A few mAbs specific for EqMHCI antigens have been well characterised (Davis et al., 1987; Donaldson et al., 1988; Kydd et al., 1991). MAbs MAC291 and CVS22 detected MHC class I molecules on the majority of equine peripheral blood leukocytes by flow cytometry and immunoprecipitated two bands of 45 and 12 kDa from equine PBL (Kydd et al., 1994; Lunn et al., 1998).

EqMHC class II molecules are composed of two non-covalently linked polypeptide chains: α (31-34 kDa), and β (26-28 kDa) (Kydd et al., 1994). An unusual characteristic of equine MHC class II antigens is that their expression is not restricted to antigen presenting cells (monocytes) but extended to a large subpopulation of equine T lymphocytes in addition to all B cells (Crepaldi et al., 1986; Lunn, 1993). This is also a feature of MHC class II in dogs and swine (Crepaldi et al., 1986; Monos et al., 1989; Holmes and Lunn, 1994). In contrast, MHCII expression in humans is restricted to antigen presenting cells (Raulet and Eisen, 1990). MAbs CZ11, CVS10, F13A.3, and CVS20 stained all B cells with a high intensity and a large subset of T cells as typical for equine MHC II markers (Crepaldi et al., 1986; Lunn, 1993). In addition, variation in expression of MHCII antigens on horse lymphocytes was detected using mAbs CZ11, EqT2, Hot214, F39.2, and CVS10 (Crepaldi et al., 1986; Barbis et al., 1994b).

A distinct characteristic of cell surface antigens is their **conservation between species** and the existence of homologues in other species for many of the important antigens described in humans. Conservation of human leukocytes differentiation molecules occurs through a variety of animal species (Ledbetter et al., 1981; Davis et al., 1987; Lunn, 1993). Establishing the homology of lymphocyte differentiation antigens in different species previously relied on the conservation of the biochemical and functional characteristics of these antigens (Ledbetter et al., 1981). The orthologues of human leukocyte differentiation molecules in most animal species examined have similar distribution patterns, biochemical composition, molecular

weight and apparent function (Ledbetter et al., 1981; Lunn, 1993). 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 and driving 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). The identity 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. Based on this conservation, **cross-reactive human mAbs** can provide the identification of homologue CD antigens in different animal species and may have valuable clinical and diagnostic uses (Reimann et al., 1994). A selection of anti-human mAbs, which were often commercially available and cross-reacted with homologous proteins of other animal species provided a highly valuable tools for veterinary medicine already (e.g. Greenlee et al., 1987; Sopp and Howard, 1997; Pedersen et al., 2002) and cross-reactivity of human and animal species monoclonal antibodies with equine leukocyte antigens was reported alike (Jacobsen et al., 1993; Pintado et al., 1995; Brodersen et al., 1998; Lin et al., 2002; Merant et al., 2003). However, many of these reports were not reproduced and others not reproducible. Accordingly, caution must be exercised and cross-reactive monoclonal antibodies thoroughly characterised against the target species, since some mAbs may identify alternative epitopes on different proteins.