II. AIM OF THE STUDY

The equine immune tool-box was established with many mAbs detecting important equine leukocyte antigens through ELAW I and II and earlier cross-reactivity studies. Despite this progress, substantial gaps remained to study equine leukocyte biology and reagents able to characterise molecules like CD14, CD25, CD34, CD45 and CD206 were not available.

The rational of the present study was to provide domestic and wild equines with cross-reacting Abs valuable to analyze leukocyte molecules. This was targeted through:

1-Analysis of a large panel of mAbs directed against various leukocyte antigens for their reactivity with equine leukocytes:

A panel of 534 mAbs against different leukocyte molecules was evaluated in this study for its cross-reactivity with equine leukocytes. 379 mAbs were submitted through the HLDA8 animal homologue section. In addition further 155 mAbs (non-HLDA8) were submitted directly from companies. The analysis was based on screening mAbs by single colour flow cytometry as a method of choice comparing the staining pattern of horse leukocytes with human leukocytes. MAbs that were defined positive here were further analyzed by two-colour flow cytometry. In particular mAbs that detected lymphocyte populations were analyzed to confirm the recognition of the appropriate subpopulation. The equine leukocyte cell lines, eCAS and EqT8888 were used to obtain more information on the analyzed mAbs. Immunoprecipitation was applied to obtain the molecular weight of equine molecules detected by cross-reactive mAbs.

2-Analysis of cross-reactive mAbs with wild equid leukocytes:

MAbs that reacted with horse (*Equus caballus*) leukocytes were further analyzed with wild equid leukocytes i.e. Somali wild ass (*Equus africanus somalicus*), Grevy's zebra (*Equus grevyi*), and Hartmann's mountain zebra (*Equus zebra hartmannae*) using one colour flow cytometry.

3-Sequence analysis and expression of CD28:

The sequence of CD28 was analyzed from members of order *Perissodactyla* including horse, Somali wild ass, Grevy's zebra, Hartmann's mountain zebra, and Greater one-horned rhinoceros (*Rhinoceros unicornis*). In addition, other wild life animals like Asian elephant (*Elephas maximus*), European bison *(Bison bonasus),* African buffalo (*Syncerus caffer*), and Nubian giraffe (*Giraffa camelopardalis*) were included. Horse CD28 cDNA was also expressed in insect cells.

4- Analysis of polyclonal antibodies to human CD28 and CD25:

Polyclonal antibodies are of multi clonal specificity and were thus used in another attempt to detect important equine CD antigens like CD28 and CD25 where none of the mAbs submitted reacted with horse leukocytes.