

Production of a pig CD34 antibody for isolation of hematopoietic stem cells

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Intensive growing conditions are often associated with a higher incidence of immune system stimulation, which can lead to a reduced growth rate. With this in mind by studying the functional development of leucocytes from pig bone marrow precursors, (hematopoietic stem cells IHSCI), we can further develop strategies directed toward enhancing the pig immune system. To date, the most effective method of HSC isolation has been through the use of a CD34 monoclonal antibody (mAb). however, as no such reagent is currently available in the pig (I lienz et al., 2002) the aim of this project was to develop antibodies against porcine CD34.

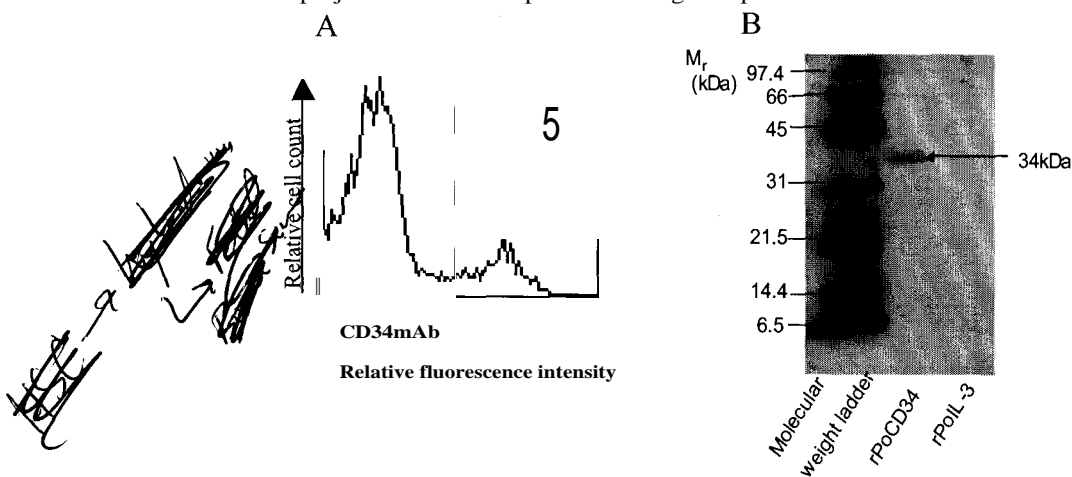


Figure 1. (A) FACS analysis of porcine BM with CD34mAb (n=5) (B) Western blot analysis of CD34mAb on rPoCD34 and rPOIL-3 (negative control) molecular weight markers.

The extracellular region of the porcine CD34 gene was cloned and inserted into prokaryotic and eukaryotic expression vectors. The prokaryotic expression vector was used to produce a recombinant porcine CD34 protein (rPoCD34) in *E. coli*. Mice, chickens and rabbits were inoculated with the rPoCD34 and the eukaryotic expression vector as a DNA inoculation. Following DNA inoculation, a boost with either the recombinant protein or porcine bone marrow cells was administered. Sera was collected and tested for reactivity against porcine bone marrow. Splens were taken from mice and hybridomas generated using Sp2 myeloma cells. Fluorescent Activated Cell Scanning (FACS) analysis was used to identify positive clones and polyclonal antibodies.

A positive hybridoma was identified that stained about 5% of the porcine bone marrow cells (Figure 1A) as well as a population of CD90+ cells, which correlated with previous observations in human and mouse CD34 studies. This mAb also bound the 34kDa rPoCD34 on western blot (Figure 1B), indicating that the antibody was against CD34. HSC were isolated using this mAb and showed stem cell enrichment in a methylcellulose assay, as seen with studies of human CD34+ cells (Verfailhe, et al., 1990).

The pig CD34 antibody has enabled the detection and isolation of pig HSC, a step that will allow studies of hematopoiesis and potentially could identify ways to enhance the pig immune system and thus pig production.

References:

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