SHORT COMMUNICATION

Monoclonal antibodies against human CD34 antigens do not cross-react with ovine umbilical cord blood cells

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Abstract

CD34 is a cell surface glycoprotein expressed by hematopoietic progenitors and endothelial cells. It is widely used in the clinic for isolation of human hematopoietic stem cells. In recent years large animals are gaining increasing importance in biomedical research for the study and therapy of human diseases. Sheep has proved to be an useful experimental model for preclinical trials in transplantation procedures. Unfortunately, the lack of specie-specific monoclonal antibodies (MABS) recognizing hemopoietic progenitor cells hampers the use of this animal in experimental hematology. The aim of this paper was to determine whether commercial monoclonal antibodies specific for human CD34 molecule could cross-react with hematopoietic progenitor cells (HPC) present in sheep umbilical cord blood (UCB). Six anti-human CD34 MABS, recognizing the three different epitope classes, were tested in flow cytometry on purified mononuclear cells (MNC) isolated from cord blood of both species. None of the MABS used in this trial seemed to be able to identify HPC from sheep UCB. These data suggest that the panel of monoclonal antibodies used for cross reactivity detection has to be expanded with recently produced reagents. Further studies should be directed towards the production of ovine specific anti CD34 MABS.

Introduction

CD34 is a transmembrane glycoprotein, member of the sialomucin family whose physiological roles are still poorly understood (Lanza *et al.,* 2001). At present, CD34 represents the only currently known cell surface antigen able to identify early hematopoietic progenitor cells of all lineages (Civin *et al.,* 1984; Krause *et al.,* 1996). The heavily glycosylated cell surface molecule gives rise to a number of different epitopes (Watt *et al.,* 1987). Monoclonal antibodies have been recognized in three classes according to their epitopes' recognition and sensitivity to enzymatic cleavage with different glycoproteases (Sutherland *et al.,* 1992). The importance of CD34 molecule for experimental hematology is well recognized (Gratama *et al.,* 1998), but availability of specific monoclonal antibodies is still limited to few animal species. The surface expression of CD34 antigens is commonly used to discriminate between progenitor cells and mature blood cells in humans (Andrews *et al.,* 1986; Berenson *et al.,* 1991; He *et al.,* 1992), nonhuman primates (Berenson *et al.,* 1988; Izawa *et al.,* 2004), rodents (Brown *et al.,* 1991; Krause *et al.,* 1994; Morel *et al.,* 1996; Garlanda *et al.,* 1997) and dogs (McSweeney *et al.,* 1998; Niemeyer *et al.,* 2001).

The use of animal models in preclinical trials is fundamental for estimating the safety and efficacy of innovative treatments such as cell and gene therapy. Large animals, rather than commonly used laboratory rodents, allow a better transposition of biomedical research results into clinical design. Sheep has been largely employed to test efficacy of stem cells transplantation procedures (Menard *et al.,* 2005; Menasche, 2005; Narayan *et al.,* 2006) and it is considered an established animal model for tissue engineering (Erggelet *et al.,* 2007; Mendelson *et al.,* 2007; Sales *et al.,* 2007). At the moment, the lack of monoclonal antibodies recognizing ovine hematopoietic progenitor cells hampered the use of this animal for developing new transplantation procedures useful in hematology disorders therapy. For this reason it is still of considerable value to test MABS defined in other species for cross-reactivity. The aim of this study was to determine by use of flow cytometry analysis whether monoclonal antibodies specific for three different epitopes of human CD34 molecule could identify hemopoietic progenitor cells in sheep umbilical cord blood.

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Materials and methods

Samples of UCB was collected from the umbilical vein of five sheep undergoing caesarian section and from five women after vaginal delivery. MNC were isolated in both species using a density gradient centrifugation (Hystopaque-1077; Sigma). Cord blood MNC were analyzed in flow cytometry for cell-surface expression of CD34 using different MABS conjugated with phycoerythrin (PE) recognizing the three different classes of human epitopes: Immu 133 (class I; IgG1, Immunotech, Beckman Coulter, Fullerton, CA, USA), Q-bend/10 (class II; IgG1, Immunotech Beckman Coulter, Fullerton, CA, USA), 563 (class II; IgG1, BD; San Jose, CA, USA), 8G12 (class III; IgG1, BD, San Jose, CA, USA), AC136 (class III; IgG1, Miltenyi Biotec, Bergisch Gladbach, Germany) and 581 (class III; IgG1, Immunotech Beckman Coulter, Fullerton, CA, USA). Immu 133, Qbend/10 and 581 MABS were purchased pooled together in liquid form (CD34 pool). An isotypic control in order to discriminate for non-specific reaction of the tested MABS (Mouse IgG1-PE, Beckman Coulter) was used. An amount of 1×10^6 cells in a 100 µL final volume of each sample were incubated in dark for 30 minutes on ice, following manufacturers suggestion for antibodies dilution: Immu 133, Q-bend/10, 563, 8G12 and 581 MABS were used at 1:5 dilution while AC136 at a dilution of 1:11. Stained cells were analyzed on a FacsCalibur (BD) flow cytometer. Thirty thousand events were acquired for each samples, and analysis were performed by Cell Quest Pro (BD).

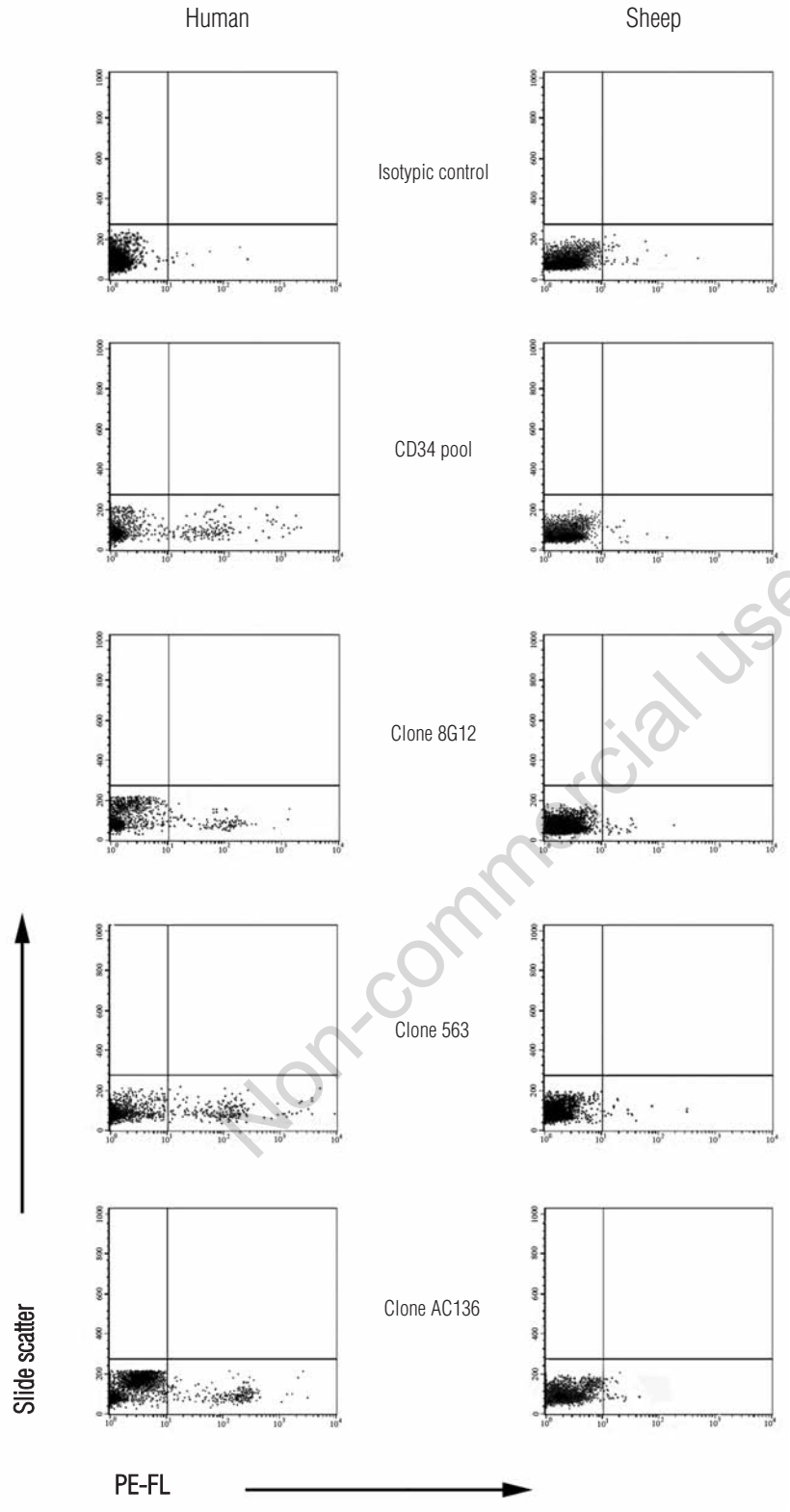

Figure 1. Cross-reaction analysis of human specific anti-CD34 monoclonal antibodies with ovine umbilical cord blood mononuclear cells. Dotplots (fluorescence two *vs* side scatter) were obtained after immunofluorescence and flow cytometric analysis. For each antibody the characteristic result obtained with one out of five human and ovine tested samples is shown.

Results and discussion

To our knowledge, this is the first attempt to identify ovine cord blood HPC using human specific anti-CD34 MABS.

In our experimental conditions, none of the tested commercial anti-CD34 MABS was able to recognize sheep cord blood HPC. On the contrary, analysis of human UCB samples, used as control, showed that a percentage of MNC ranging from a low of 0,5% to a high of 2% were CD34 positive, in accordance with data reported by other authors (D'arena *et al.,* 1996; Belvedere *et al.,* 1999; Ende *et al.,* 2001) (Figure 1).

The anti-human CD34 MABS tested in flow cytometry did not prove to be cross-reacting with ovine UCB cells, suggesting that CD34 molecule primary and tertiary structure differs in the two species. Indeed, neither the use of MABS directed to class I and II linear epitopes, nor MABS directed to class III conformational epitopes resulted in a positive reaction.

The choice to test human-specific products was mainly determined by the availability of several anti-human MABS clones directed against the three different CD34 epitopes. However, the possibility that commercially available MABS produced for other species could cross-react with ovine cells should be considered. Recently, Sakurai *et al.* (2006) reported the production of two monoclonal antibodies against bovine CD34. It might be expected that anti-CD34 MABS raised against bovine antigen cross-reacted with ovine cells. Unfortunately, we were not able to test these bovine specific MABS since they still were not commercially available.

Conclusions

In conclusion, our findings suggest that the number of monoclonal antibodies for cross-reactivity detection need to be extended with those of recent production and that further studies should be directed toward the production of specific ovine anti-CD34 MABS.

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