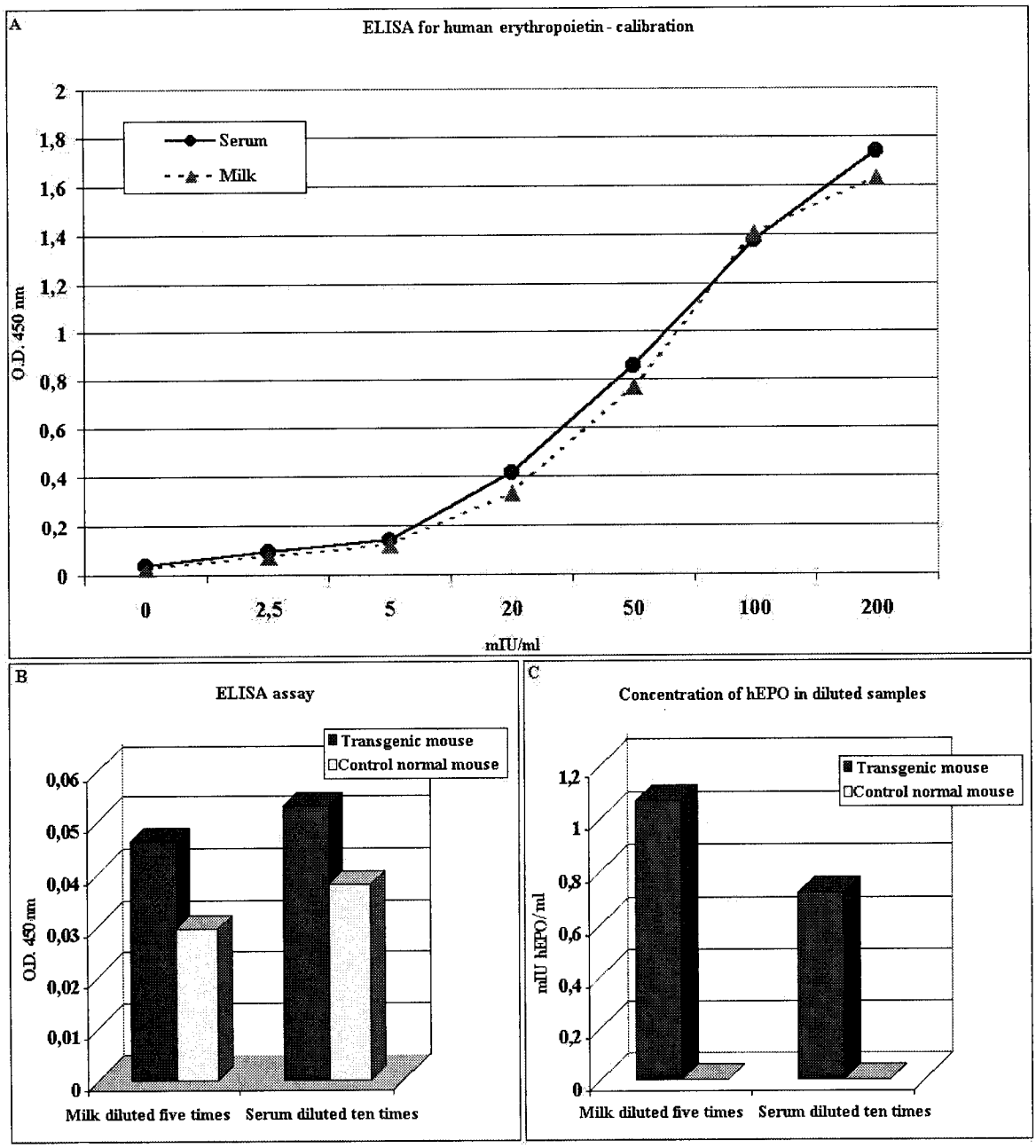

Fig. 5. Determination of hEPO concentration by ELISA testing in the milk and blood of a transgenic mouse carrying the hybrid gene *rWAP/hEPO*. A standard curve was obtained by adding hEPO into the mouse blood and milk. B: Signals of absorbance in diluted milk and blood samples of the transgenic mouse carrying the hybrid gene *rWAP/hEPO* and of a control normal mouse. C: Levels of hEPO in diluted samples of the milk and blood of the transgenic mouse carrying the hybrid gene *rWAP/hEPO*.

Discussion

Microinjection of foreign DNA into the pronuclei of mouse ova is the most common method for generation of transgenic animals. In our study, two constructs of different sizes, phEBS-HB, carrying the *hEPO* gene, and prWhEBS-BNX, carrying the *rWAP/hEPO* hybrid gene, were microinjected into the pronuclei of ova. At the beginning, we used vector phEBS-HB to optimize the techniques for generation of transgenic mice and also to test the specificity of primers and their capability to identify *hEPO* transgenic mice using PCR. Later, we used the chimaeric *rWAP/hEPO* gene construct to verify the ability of the *rWAP* 5' flanking region (8.5-kb) to direct the expression of *hEPO* into the mammary gland of the host animal. Under normal conditions, *WAP* gene expression is restricted to the mammary gland cells only. Although the upstream region of the *WAP* gene has been described to be a very efficient DNA regulatory element for targeted expression in the

mammary gland, it seems to be clear that the resulting level of transgenic expression remains to also be dependent on the site of integration (Devinoy et al., 1994). This is mainly due to differences in the chromatin structure surrounding the site of transgene integration and is also a result of the number of introduced gene copies. Mutual interactions of hormone responsive elements located within 5' untranslated sequences of the *WAP* gene together with the regulation elements located within the structural part of the transgene may result in a new variant of expression (Brem et al., 1991; Gunzburg et al., 1991). Thus, different structural genes fused together with *WAP* regulatory sequences can modify the tissue-specific expression pattern in comparison to the endogenous gene expression profile.

In previous works (Gordon et al., 1987; Pittius et al., 1988; Tomasetto et al., 1989; Devinoy et al., 1991a; Gunzburg et al., 1991; Bischoff et al., 1992; Velander et al., 1992; Devinoy et al., 1994; Limonta et al., 1995a; Limonta et al., 1995b; Rodriguez et al., 1995; Thepot et al., 1995; Massoud et al., 1996; Aguirre et al., 1998), the upstream region (6.3-kb) of the *rWAP* gene was found to be sufficient to target high transgene expression into the mammary gland. However, when the same DNA fragment was linked to the *hEPO* gene, the expression was currently low (Rodriguez et al., 1995; Massoud et al., 1996; Aguirre et al., 1998). Transgenes are known to be much better expressed when their structural part is a native gene, with their introns, rather than cDNA. However, the introns in the *hEPO* gene may contain silencers, which can reduce the expression in non-renal cells. In order to achieve specific and high expression of *hEPO* in the mammary gland of the mouse, we used the upstream promoter sequence (8.5-kb) of the *rWAP* gene, introduced into the prWhEBS-BNX construct, to direct expression of the *hEPO* gene.

Similar constructs, but with a shorter part of the *rWAP* promoter, have been recently used in mice and rabbits to express the *EPO* gene in the mammary gland (Rodriguez et al., 1995; Massoud et al., 1996; Aguirre et al., 1998). In our case, samples of the milk extract and blood serum of transgenic mice were assayed by commercial ELISA. The milk extract contained 5.3 mIU of the hEPO/ml, thus showing that the cloned *rWAP* promoter was able to direct synthesis of the *hEPO* gene into the mammary gland of the transgenic mouse.

The main goal of this study was to verify the function of the chimaeric gene *rWAP/hEPO in vivo* using the transgenic approach.

It is possible that increased expression of the *rWAP/hEPO* fusion gene, and recombinant hEPO production, will be achieved in transgenic rabbits, where rabbit-regulatory elements of the *WAP/hEPO* construct are believed to be more efficient to control and restrict expression of the transgene.

Our data also show that the expression of the *hEPO* transgene in a transgenic animal is not strictly limited to the mammary gland. We found ectopic expression of the transgenic *hEPO* product in the blood. This corresponds to previously obtained results where the *rWAP* promoter caused ectopic expression of the *hEPO* gene, resulting in a deleterious effect of *hEPO* in transgenic rabbits (Massoud et al., 1996). At this moment we cannot exclude the role of other genetic factors affecting *EPO* expression in the transgenic animal, including the combinatory effect of the integration site, *EPO* intragenic elements and the number of gene copies. An additional modification of the regulatory recombinant sequences can be performed to optimize the specificity and level of the desired therapeutic glycoprotein production.

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