

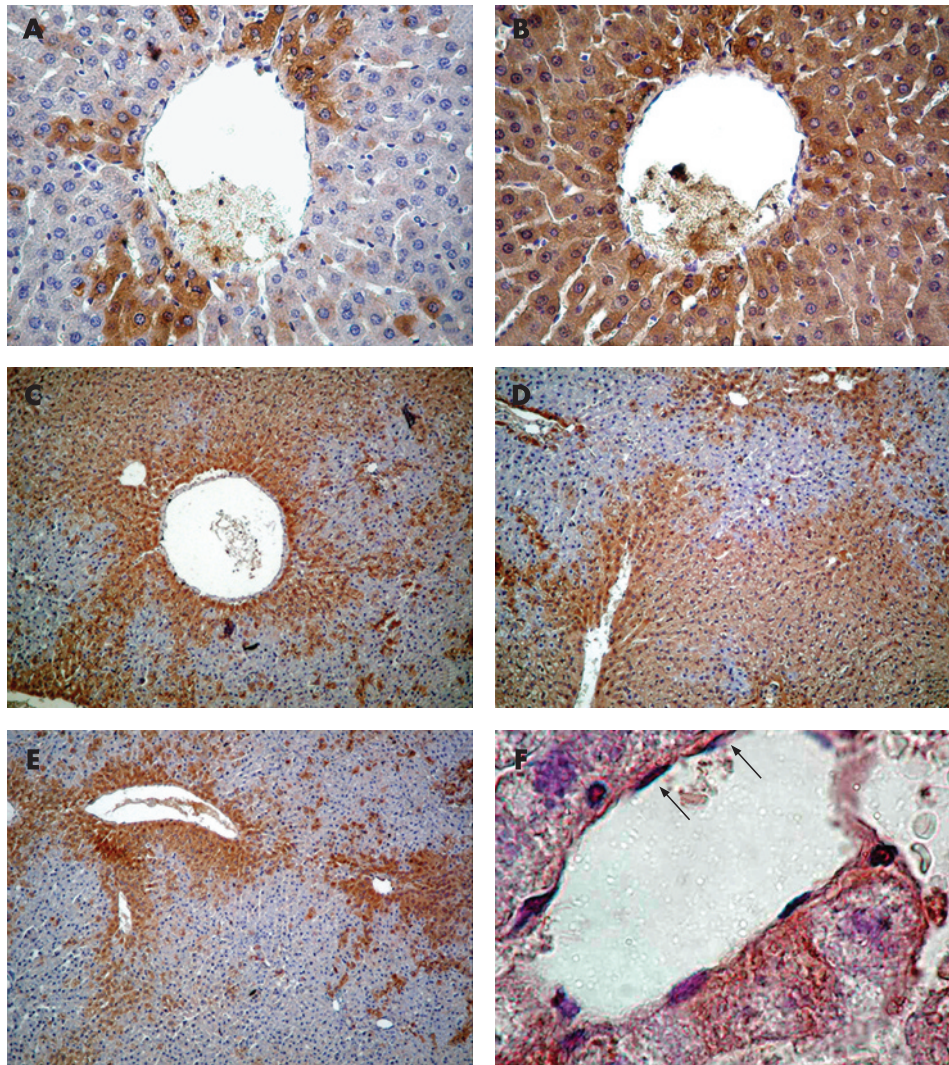
**Figure 4** Percentage of green fluorescent protein (GFP) positive hepatocytes (Hcs) one month after GFP positive bone marrow derived cell (BMDC) transplantation, as a function of the number of infused cells (group F;  $n=8$ ).

three bands (511, 67, and 12 bp, the last one not visible). In Gunn j/j rats, which received infusion of BMDCs from non-affected, wild type animals, four bands are visible, including those derived from digestion of the normal enzyme.

## DISCUSSION

There has been much interest in cell therapy for liver metabolic disorders in the last decade, and several investigators have reported a significant clinical improvement following hepatocyte transplantation as an alternative to liver transplantation.<sup>9</sup> Major limitations are the limited availability of human hepatocytes and the limited long term efficacy. In theory, the use of BMDCs as an alternative to hepatocytes could solve both problems. The present work indicates first, that a significant parenchymal repopulation with hepatocyte-like cells derived from BMDCs can be induced even in metabolic disorders not associated with increased parenchymal cell turnover; second, that probably such repopulation is mostly due to differentiation rather than to cell fusion; and third, that such repopulation results in partial correction of a congenital hepatic metabolic disorder.

The extent of parenchymal repopulation with exogenous bone marrow derived, GFP positive hepatocyte-like cells averaged 0.4% in the liver lobes submitted to I/R 72 hours after infusion, but it increased by more than 20-fold after one month, suggesting proliferation of the exogenous cells. The histological finding showing large clusters of GFP positive hepatocytes supports this hypothesis. Continuous propagation of stem/progenitor cells in the absence of ongoing liver damage can be driven by cell-cell competition, with increased proliferation and reduced apoptosis of the transplanted cells when



**Figure 5** Bone marrow derived cells (BMDCs) isolated from green fluorescent protein (GFP) positive transgenic rats acquire morphological features and phenotypic markers of differentiated hepatocytes after transplantation into liver injured Lewis recipients (group F). Serial sections of livers removed four weeks after cell transplantation were stained for GFP (A) and for CK-18 (B). (C-E) Extensive repopulation of liver parenchyma with GFP positive hepatocyte-like cells four weeks after transplantation of  $10 \times 10^6$  GFP positive BMDCs (group F). Large clusters of GFP positive hepatocyte-like cells are mainly located in proximity of blood vessels. (F) GFP positive endothelial-like cells (arrows) are also visible in the ischaemic lobes. (Original magnification: (A), (B)  $\times 20$ ; (C), (D), (E)  $\times 10$ ; (F)  $\times 40$ .)