Severe acute liver failure, even when transient, must be treated by transplantation and life-long immune suppression. Treatment could be dramatically improved by bioartificial liver (BAL) support, but a shortage of human hepatocytes to construct a BAL limits its application. To generate an alternative source of cells for BAL support, we differentiated ES cells into hepatocytes by co-culture with a combination of human liver non-parenchymal cell lines and fibroblast growth factor-2, human activin-A, and hepatocyte growth factor. Functional hepatocytes were isolated using albumin promoter-based cell sorting. ES-derived hepatocytes expressed liver-specific genes, secreted albumin, and metabolized ammonia, lidocaine, and diazepam. Treatment of 90% hepatectomized mice with a novel subcutaneously implanted BAL seeded with ES-derived hepatocytes or primary hepatocytes dramatically improved liver function and prolonged survival, while treatment with a BAL seeded with control cells did not. After seeding in the BAL, ES-derived hepatocytes developed characteristics nearly identical to those of primary hepatocytes.