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Biochemical and Biophysical Research Communications 329 (2005) 337–344
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Hepatic progenitor cell resistance to TGF-β1's proliferative and apoptotic effects ☆

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Received 29 December 2004

Abstract

The success of hepatocellular therapies using stem or progenitor cell populations is dependent upon multiple factors including the donor cell, microenvironment, and etiology of the liver injury. The following experiments investigated the impact of TGF-β1 on a previously described population of hepatic progenitor cells (HPC). The majority of the hepatic progenitor cells were resistant to endogenously produced TGF-β1's proapoptotic and anti-proliferative effects unlike more well-differentiated cellular populations (e.g., mature hepatocytes). Surprisingly, in vitro TGF-β1 supplementation significantly inhibited de novo hepatic progenitor cell colony formation possibly via an indirect mechanism(s). Therefore despite the HPC's direct resistance to supplemental TGF-β1, this cytokine's inhibitory effect on colony formation could have a potential negative impact on the use of these cells as a therapy for patients with liver disease.

Keywords: Liver; Hepatocyte; Cytokines; Apoptosis; Stem cells; Progenitor cell; Caspase; Proliferation

The excitement surrounding hepatic progenitor (and stem) cells has grown exponentially in the past few years with the isolation and characterization of several distinct hepatic-derived populations [1–10]. We have isolated a hepatic progenitor cell (HPC) population that is unique because it arises from the naïve adult liver. These small mononucleated cells proliferate and differentiate into cells of both hepatic and biliary lineage [11]. As investigators develop cell therapy strategies based on novel stem cell populations, it is imperative to determine how the host (or recipient) microenvironment will impact progenitor or stem cell engraftment, proliferation, and subsequent cellular function. One potential mechanism involves paracrine signaling via locally produced cytokines.

The effects of several liver-related cytokines on mature hepatocytes have previously been studied. Transforming growth factor β (TGF-β) is a natural growth inhibitor for epithelial-derived cells that initiates its signaling by binding and activating TGF-β receptor types I (RI) and II (RII). TGF-β has a known antiproliferative effect on hepatocytes, as its administration at the time of liver resection has been shown to delay hepatocyte proliferation [12,13]. Even at picomolar concentrations, TGF-β has been shown to inhibit the proliferative effect of other cytokines (e.g., epidermal growth factor) [14]. In the uninjured adult liver, sinusoidal endothelial cells and Kupffer cells have relatively high constitutive levels of messenger RNA for TGF-β1. This is in contrast to stellate cells that express very little TGF-β in the basal

☆ American Liver Foundation Liver Scholar Award (D.A.G.); NIH DK59302 (D.A.G.); Faculty Development Award from the American College of Surgeons (D.A.G.); University Research Council Grant (D.A.G.) funded this work.
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doi:10.1016/j.bbrc.2005.01.129

state, but become the principal source of TGF-β production after injury [13]. Hepatocytes of the normal adult liver express undetectable levels of TGF-β1 but they respond to TGF-β1 produced by nonparenchymal liver cells by inhibiting DNA synthesis and inducing apoptosis [15,16]. Accordingly, TGF-β1 has been proposed to control homeostasis of liver mass in vivo through its contribution to the termination of hepatic proliferation upon liver regeneration and elimination of surplus hepatic cells during adaptive liver growth [15,17].

Activated TGF-β1 has been shown to inhibit hepatocyte proliferation and induce apoptosis of both adult and fetal differentiated hepatocytes [14,18–25]. Somewhat surprisingly there is a subpopulation of fetal rat hepatocytes that are resistant to exposure to TGF-β. This subpopulation represents 40–50% of the total fetal rat hepatocyte population. Further isolation of this TGF-β resistant cell population showed that these cells require mitogens to grow and maintain their response to TGF-β1 in terms of growth inhibition [26,27]. In fetal hepatocytes, TGF-β produces a 10-fold increase in caspase-3 activity which is attenuated in the presence of epidermal growth factor [28]. The impact of TGF-β1 on bipotent progenitor or stem cell populations remains unstudied to date. The focus of these experiments is to evaluate TGF-β1's effects on an adult-derived hepatic progenitor cell population.

Materials and methods

Mice. C57Bl/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All animals were maintained on a rodent chow under a constant day/night cycle. Six- to eight-week-old mice were used in all experiments. All care and use of animals was approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill in accordance with the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Reagents in these experiments were from Sigma, St. Louis, MO, unless otherwise stated.

Liver cell isolation and culture. Liver cells were isolated using a modification of the two-stage liver perfusion technique described by Seglen [29]. The hepatic progenitor cells were separated from mature hepatocytes using gravity separation and centrifugation. The fraction containing hepatic progenitors was plated on collagen I coated dishes at a cellular density of $6–8 \times 10^5$ cells per 35-mm dish in Dulbecco's modified Eagle's medium (DMEM) plus 10% fetal bovine serum (FBS), 20 mM hepes, 10 mM nicotinamide, 1 mM ascorbic acid 2-phosphate, 1 μM dexamethasone, 0.5 mg/L ITS solution, 30 mg/L proline, 100 mg/L antibiotic solution, and 10 ng/ml epidermal growth factor (EGF). The cells were cultured in a 5% CO_2/95% room air incubator at 37 °C and the media were changed every 3 days. Full details of the isolation and characterization of the hepatic progenitor cells have been previously described [11].

ELISA. The concentration of TGF-β1 in the cell culture media was measured by enzyme-linked immunosorbent assay (ELISA). Media were collected from control dishes at days 4, 7, 11, 14, and 21, and placed in cold storage at −20 °C until processing. The samples were thawed only once prior to ELISA. The samples were analyzed by ELISA according to the manufacturer's protocol for human TGF-β1 (Promega, Madison, WI). The concentrations of active TGF-β1 were measured by acidifying and subsequently neutralizing the media as per the manufacturer's protocol and active TGF-β1 concentrations within the culture supernatants were calculated from standard curves generated with each assay.

Cytokine administration. TGF-β1 was reconstituted as per the manufacturer's protocol with 0.1% BSA and added to the media for its final concentration. TGF-β1 was added to the culture media beginning at day 0 (the day of isolation) to evaluate its impact on hepatic progenitor cells prior to de novo colony formation. All further media changes included the same concentration of TGF-β1. TGF-β1 was used in concentrations ranging from: 0.005 to 5 ng/ml. Anti-TGF-β1 (range: 0.01–1 μg/ml) was used in a separate group of experiments to determine if cytokine binding would prevent its interaction with the TGF-β1 receptor on hepatic progenitor cells.

Plasmids. The p3TP-LUX plasmid is a chimeric TGF inducible reporter containing multimerized TGF binding elements inserted upstream of the human plasminogen activator 1 gene. This construct contains three CAGA elements that are essential and sufficient for induction by TGF-β. This assay tests for TGF-β responsive genes with SMAD binding elements (SBEs) in the promoter. This plasmid was a kind gift of Dr. Kevin Behrns at the University of North Carolina at Chapel Hill.

Proliferation and apoptosis assays. Hepatic progenitor cell proliferation was determined using bromodeoxyuridine (BrdU), which is incorporated in place of thymidine during the S-phase of cycling cells. Anti-BrdU-FITC (BD PharMingen, San Diego, CA) was used to stain proliferating cells as per the vendor's recommendation. Analysis was performed by flow cytometry on a FACScan (Becton–Dickinson Immunocytometry Systems), interfaced to Cytomation, Cicero data acquisition systems.

Cellular apoptosis was analyzed by staining with Annexin V (BD PharMingen). Annexin V is used to quantitatively determine the percentage of cells undergoing apoptosis by relying on the property of cells to lose membrane asymmetry in the early phases of apoptosis. Annexin V has a high affinity for phospholipid phosphatidylserine and in conjunction with propidium iodide (PI) can distinguish viable from nonviable cells. Cells were washed and cultured with 5 μl annexin V-FITC and 10 μl PI. As per the vendor's recommendation, the percentage of cells undergoing apoptosis is determined by subtracting from those cells that are Annexin V(+), the cells that are also PI(+) divided by the total cell population.

Immunofluorescent studies. For immunofluorescence detection of caspase 3, cells were washed twice with PBS and fixed in 1:1 acetone:methanol for 10 min. Cells were exposed to the primary antibody against caspase 3 for 1.5 h at room temperature, washed twice in PBS, and then exposed to the conjugated secondary antibody for 1.5 h at room temperature. Cells were visualized on a Zeiss Axiocarvert 100 direct view real-time white light confocal phase fluorescence microscopy system with an axiocam digital camera. Images were acquired at 488 nm laser excitation for FITC-conjugated antibodies. Digital images were processed using Adobe Photoshop 6.0 (San Jose, CA).

Inhibition of caspase 3 activity. Hepatic progenitor cells in culture were identified after early colony formation was identified and the medium was switched to TGF-β1 and supplemented with Z-DEVD-FMK (caspase-3/CPP32) a caspase 3 inhibitor (Calbiochem, La Jolla, CA). After an additional 18 h in culture, the rate of apoptosis of the hepatic progenitor cells was determined as previously described.

Transient transfection and luciferase assays. Each cell culture dish was transfected with 0.4 μg of the plasmid per plate. Four hours after transfection, the cells were washed twice with DMEM and then cultured with 10% DMSO. The cells were harvested 12–24 h after transfection and exposed to one freeze–thaw cycle in Reporter Lysis Buffer (Promega). Firefly luciferase activity was determined using the dual luciferase assay system (Promega) and normalized to total cell protein.

Statistical analysis. Data are expressed as means ± SD. Statistical analysis was performed using Student's *t* test and statistical differences were considered significant at $p < 0.05$. Analysis was performed using GraphPad InStat Software version 3.05 (San Diego, CA).

Results

TGF-β1 in vitro production with hepatic progenitor cells

Our initial experiments were designed to study the impact of TGF-β1 on HPC by examining basal production of active TGF-β1 during the first 3 weeks of primary cell culture. This is a time period when we routinely observe hepatic progenitor cell proliferation and de novo colony formation. Hepatic progenitor cell colonies were established using the following methodology. A cellular population of the *adult* liver was isolated containing small parenchymal and nonparenchymal cells through a combination of mechanical and enzymatic digestion steps [11]. This method of mechanical separation leads to a cell fraction that contains the HPC and a subpopulation of contaminating nonparenchymal cells. The composition of this cellular fraction includes Kupffer cells comprising 0.4% of the cell population, stellate cells making up 13% of the population, CD11c expressed on 4% of the cells, and 12% of the cell population expressing CK7 (a marker of glandular epithelium) [11]. During the first 3 weeks of culture, the number of hepatic progenitor colonies increased from 0 at day 4, to 4.2 ± 2.2 colonies/dish at day 7, to 94.25 ± 11.35 colonies/dish at day 21 of culture. During that same period of time, the amount of active TGF-β1 produced in culture varied from 26.9 ± 2.6 pg/ml/24 h at day 4 to a peak of 46.6 ± 4.3 pg/ml/24 h at day 21 (Fig. 1). It is presumed that the nonparenchymal population is the source of TGF-β1 in this culture. These experiments demonstrate no correlation between the concentration of active TGF-β1 produced in the in vitro system and the proliferation of hepatic progenitor cells (defined as colony formation).

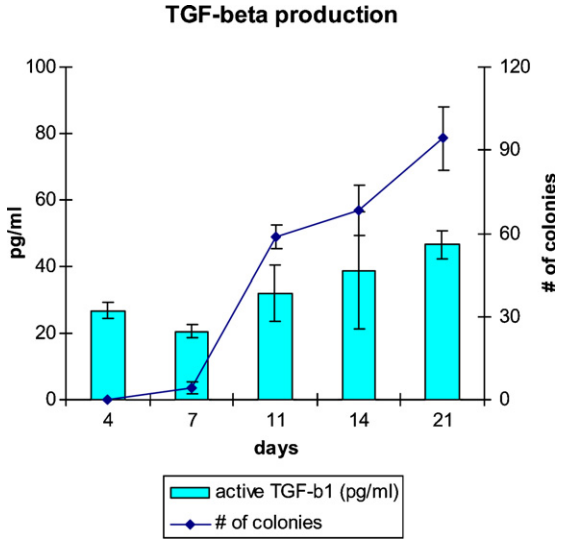

Fig. 1. Hepatic progenitor cells were isolated and established in culture. Media were collected from a minimum of 5 dishes per time point and assayed by ELISA for active TGF-β1 production per 24 h per dish. Hepatic progenitor colonies were counted per dish at each time point. Results shown are the average of three experiments.

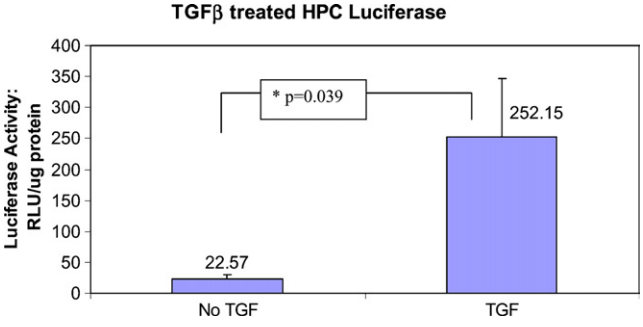

Fig. 2. The effect of 5 ng/ml TGF-β1 on the activity of the transfected p3TP-LUX plasmid, a TGF-β inducible reporter containing three TGF-β binding elements, in hepatic progenitor cells. The cells were exposed to TGF-β1 for 18 h. Luciferase activity is expressed as relative luciferase expression per microgram of protein. Data are presented as means + SD of three experiments.

TGF-β receptor and hepatic progenitor cells

To determine if our HPC population had an intact TGF-β receptor signaling pathway, we used a p3TP-Lux plasmid. It is known that signaling via TGF-β receptors leads to phosphorylation of proteins of the SMAD family. This phosphorylation causes SMADs to move into the nucleus, where they directly control gene expression [30]. We used an assay which expresses luciferase as a signal of SMAD3 phosphorylation, a marker of a competent TGF-β intracellular signaling pathway. To study this pathway, we transfected colonies of HPC with a p3TP-LUX plasmid [31]. HPC were established in culture for 2 weeks and 5 ng/ml TGF-β1 was added to the treatment arm. The colonies were then assayed for luciferase expression after 18 h of culture. We demonstrated a 10-fold increase in the relative amount of luciferase expressed in the TGF-β1 treated cells versus untreated HPCs ($p = 0.039$) (Fig. 2). These results suggest that the TGF-β signaling pathway is competent within the HPC colonies.

TGF-β1 and hepatic progenitor cell colony formation

The next experiments analyzed the effect of TGF-β1 supplementation on de novo formation of hepatic progenitor cell colonies. Our routine culture conditions were supplemented with TGF-β1 at varying concentrations (range: 0.005–5 ng/ml) beginning at day 0 and

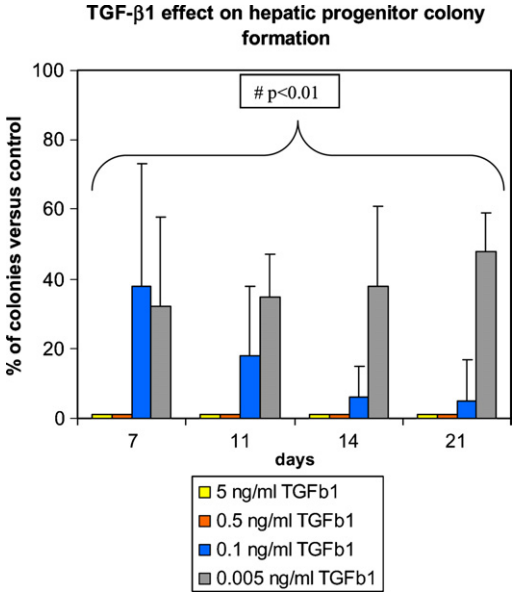

Fig. 3. Hepatic progenitor cells were isolated and cultured under routine conditions with various concentrations of TGF-β1 supplementation. The mean number of hepatic progenitor colonies/10-high power fields on treated dishes was counted at select time points and compared with the mean number of hepatic progenitor colonies/10-high power fields from control dishes.

continuing through day 21 of culture. Hepatic progenitor cell colony formation was analyzed in control and experimental dishes and the number of hepatic progenitor colonies/10-high power fields (20×)/dish was counted in each arm of the experiment. A minimum of three dishes was included for analysis. Colony formation was completely inhibited with the addition of TGF-β1 at the higher concentrations (0.5, 1 [data not shown], and 5 ng/ml) (Fig. 3). TGF-β1 supplementation at lower concentrations (e.g., 0.1 and 0.005 ng/ml) demonstrated partial inhibition of hepatic progenitor colony formation with 5% of colonies forming in the 0.1 ng/ml concentration (at day 21) and 48% of colonies forming in the 0.005 ng/ml concentration (relative to control dishes) (Fig. 3). Statistical analysis of the inhibition of the percentage of HPC colony formation versus control dishes was significant at all of these concentrations (range: 0.005–5 ng/ml TGF-β1; $p < 0.01$).

In an experiment designed to neutralize the effect of endogenously produced TGF-β1, we cultured hepatic progenitor cells with anti-TGF-β1 (0.01, 0.1 or 1 μg/ml) for 21 days to evaluate colony formation. In these experiments, the cells remained viable and the number of colonies formed was no different in number when compared with hepatic progenitor colonies in the control dishes ($p = \text{NS}$) (data not shown).

HPC proliferation and TGF-β

TGF-β's antiproliferative action on mature hepatocytes has been documented using the partial hepatectomy model in rats where post-liver resection hepatocytes exhibit a transient increase in TGF-β2 and TGF-β3 as part of the early response [13,32]. To further investigate TGF-β1's impact on hepatic progenitor cells, we analyzed hepatic progenitor cell proliferation using BrdU incorporation. HPC plated on 35-mm tissue culture dishes were observed until the formation of early colonies was visually established. This typically occurs by the end of the first week of culture. TGF-β1 was supplemented to the media (5 ng/ml) and BrdU was added to the culture to determine the impact of the cytokine on HPC proliferation. Cells were incubated for 40 h and subsequently stained with anti-BrdU-FITC. The cells were assayed by flow cytometry. There was no difference in the percentage of proliferating HPC after adding 5 ng/ml TGF-β1 to hepatic progenitor cell colonies (control 45.7% versus 43.1% in the treated cells; $p = \text{NS}$) (Fig. 4).

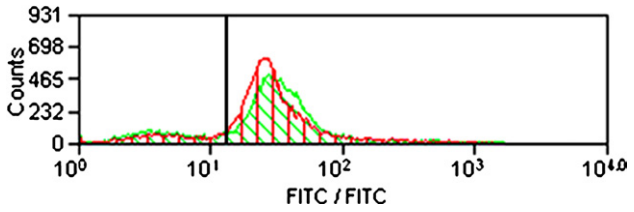

Fig. 4. BrdU staining demonstrating proliferation of hepatic progenitor cells in 1-week-old cultures. Control dishes (red, vertical bars) 45.7% proliferation and 5 ng/ml TGF-β1 supplemented dishes (green, angled bars) 43.1% proliferation. Solid vertical bar represents negative control. FACScan analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

HPC apoptosis and TGF-β

Based on the finding that TGF-β1 did not demonstrate an anti-proliferative effect on the HPC we wanted to examine its pro-apoptotic effect. TGF-β1 is known to have a dramatic impact on adult and fetal hepatocytes via a pro-apoptotic pathway [13,24,25,33,34]. We used an Annexin V assay and indirect immunofluorescent analysis to identify cells undergoing early apoptotic changes. Expanding HPC colonies were supplemented with 5 ng/ml TGF-β1 and the cells were subsequently stained for Annexin V and propidium iodide 18 h after TGF-β1 supplementation. Hepatic progenitor cells cultured with TGF-β1 for 18 hours underwent apoptosis at a rate of $1.1 \pm 2.7\%$ in standard culture conditions versus $2.5 \pm 3.5\%$ in the TGF-β1-treated cells (Fig. 5). These experiments were performed without EGF in the culture medium as EGF can inhibit TGF-β's apoptotic effect [26]. Additional experiments performed with EGF supplementation in the culture medium demonstrated comparable results (data not shown). In compar-

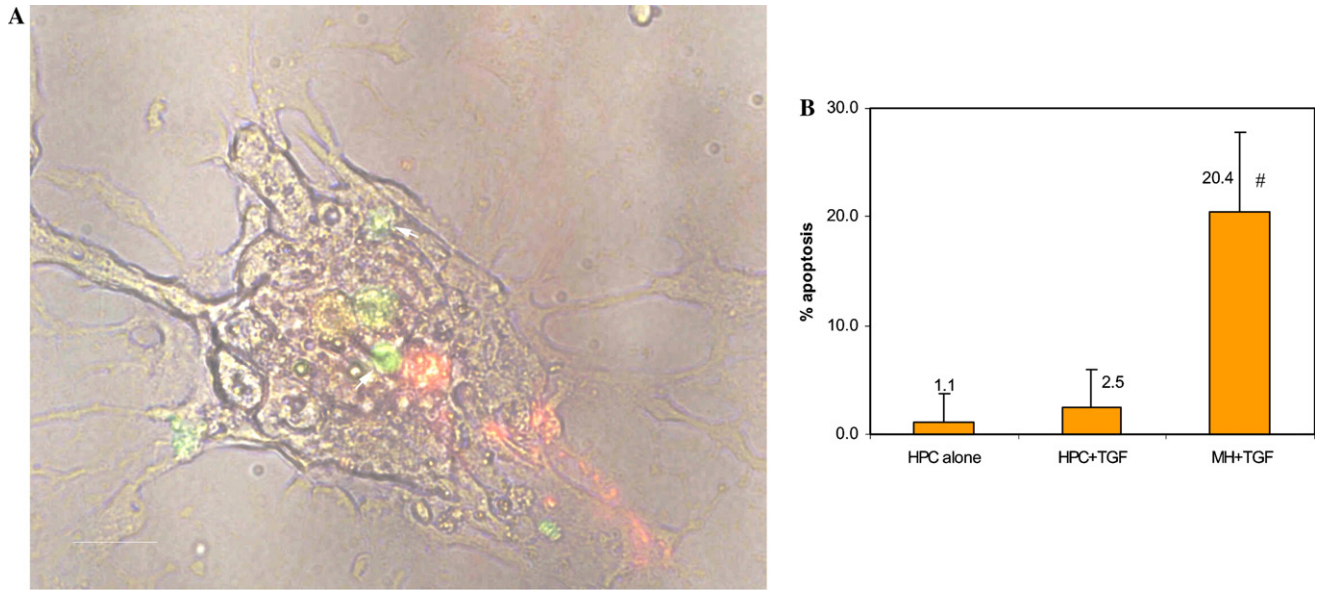

Fig. 5. Hepatic progenitor cells were established in culture and the experimental group was treated with 5 ng/ml TGF-β1 for 18 h. Cells were stained with Annexin V (green) and propidium iodide (red) to distinguish apoptotic from dead cells. Cells marked with arrows are apoptotic (A); scale bar = 20μ. The percentage of apoptotic cells was based on Annexin V+ cells/total cells/10 hpf/experiment. Each experiment was performed >3 times. Comparison arms are hepatic progenitor cells alone and mature hepatocytes treated with TGF-β1 × 18 h (B). ($^{\#}p < 0.0001$.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

ison, mature hepatocytes under similar culture conditions responded to TGF-β1 supplementation by undergoing apoptosis at a rate of 20.4%. The slight difference in the rate of apoptosis between the HPC cultured under routine conditions versus those exposed to 5 ng/ml TGF-β1 was not significant unlike the rate of apoptosis seen with the mature hepatocytes ($p < 0.001$). Experiments were performed with varying duration of incubation (12–72 h) with TGF-β1 and the results were comparable to the 18-h experiments (data not shown).

Caspase 3 activity and hepatic progenitor cells

To further investigate the intracellular signaling pathways of HPC, we studied caspase 3 activation. Hepatic

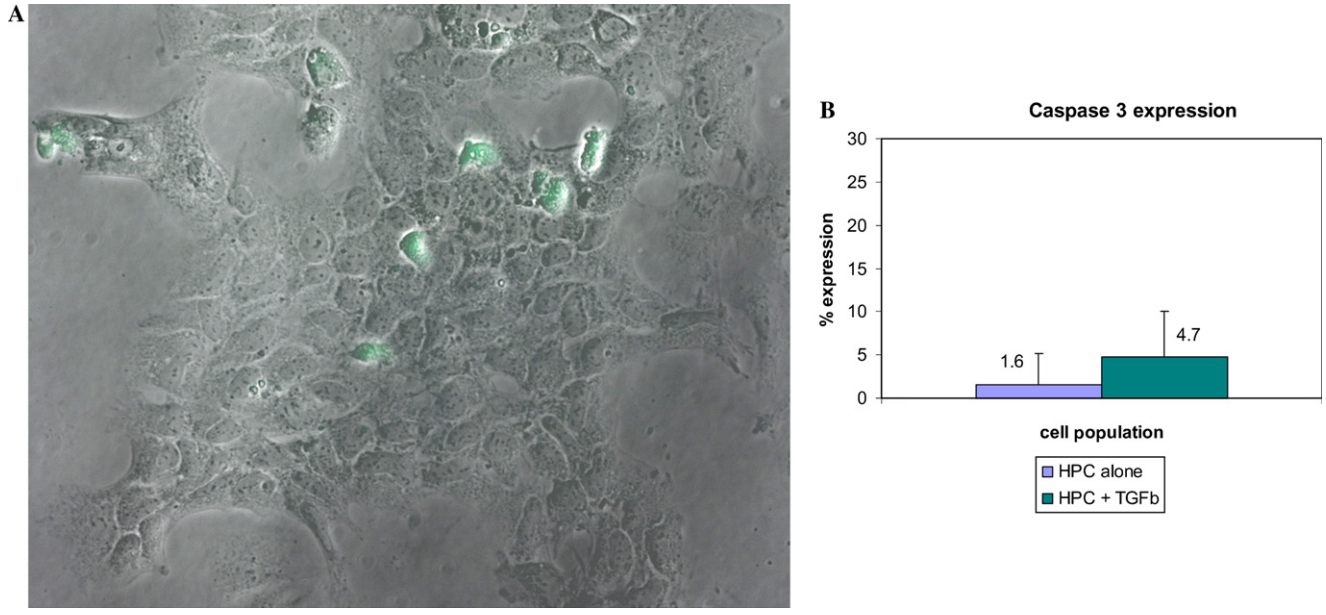

Fig. 6. Caspase 3 positive hepatic progenitor cells were cultured in routine culture conditions or with 5 ng/ml TGF-β1 (A), 200× magnification. Percentages reflect the number of positive staining cells/total cells in 10-high power fields/dish (B). These results represent a minimum of three experiments.

progenitor cells were established in culture under the previously mentioned growth conditions. After proliferating colonies were identified, the culture medium was supplemented with 5 ng/ml TGF-β1 and the cells were stained with a caspase 3 antibody 18 h after TGF-β1 supplementation. Cells were analyzed by indirect immunofluorescence and the percentage of caspase 3 positive cells was determined/10-high power fields/dish. Eighteen hours of in vitro culture with 5 ng/ml TGF-β1 increased the number of hepatic progenitor cells expressing caspase 3 from $1.6 \pm 3.6\%$ to $4.7 \pm 5.4\%$ (Fig. 6).

Caspase 3 regulated apoptosis of hepatic progenitor cells

Since the previous experiment demonstrated a greater increase in caspase 3 positive cells after TGF-β1 supplementation, compared with the percentage of apoptotic HPC we wanted to further investigate the role of caspase 3 activation on HPC. HPC were cultured in the presence of a caspase 3 specific inhibitor ± TGF-β1. The presence of Z-DEVD-FMK alone, a caspase-3 inhibitor, had an insignificant effect on hepatic progenitor cell apoptosis compared with HPC alone ($1.2 \pm 1.8\%$ vs. $1.1 \pm 2.7\%$, respectively). When HPC were cultured with TGF-β1 in the presence of Z-DEVD-FMK, the percentage of cells undergoing apoptosis was comparable to control HPC ($1.8 \pm 3\%$ vs. $1.1 \pm 2.7\%$) (data not shown).

Discussion

The de novo isolation of hepatic progenitor cells from an uninjured liver is challenging due to the lack of antigenic markers characterizing these cells [11]. Our isolation process generates a mixed population including hepatic progenitor cells and nonparenchymal cells from the liver thereby providing a cellular milieu that is a microcosm of the hepatic environment. Therefore, this in vitro model provides the opportunity to study the effects of various liver-related cytokines, like TGF-β, on the hepatic progenitor cell population.

It has already been established that the mechanisms involving hepatic regeneration are primarily driven by compensatory hyperplasia of the mature hepatocytes [35]. A variety of factors are induced during hepatic regeneration including TGF-β, produced by nonparenchymal liver cells. It is presumed that this cytokine plays a role in controlling liver mass, either through a growth inhibitory effect or through an apoptotic process [13,36]. What remains unclear is the mechanism by which alternative cellular populations (e.g., hepatic progenitor cells) would participate in this regenerative process (either through proliferation of a host cell population or by exogenous cell therapy) when replication of the mature hepatocyte population is inadequate for cellular reconstitution of the liver. Critical to determining this role for progenitor cells includes evaluating the host microenvironment as it relates to the cell population of interest. The previous experiments were designed to address the impact of a liver-derived cytokine, TGF-β1, on proliferation and viability of a bipotent hepatic progenitor cell.

Transforming growth factor β (TGF-β), a natural growth inhibitor for epithelial-derived cells, initiates its signaling by binding and activating TGF-β receptor types I (RI) and II (RII). Hepatocytes of the normal adult liver express undetectable levels of TGF-β1 but they respond to TGF-β1 produced by nonparenchymal liver cells through inhibition of DNA synthesis and induction of apoptosis [15,16]. Accordingly, TGF-β1 has been proposed to control homeostasis of liver mass in vivo through its contribution to the termination of hepatic proliferation upon liver regeneration and elimination of surplus hepatic cells during adaptive liver growth [15,17]. In primary hepatocyte and fetal hepatocyte cultures, TGF-β1 inhibits DNA synthesis from normal and regenerating livers by blocking the transition from the G_1 to S-phase of the cell cycle [18,24,37]. Additional studies looking at the in vitro effect of TGF-β on regenerating hepatocytes demonstrate a similar antiproliferative effect [36].

With our in vitro model of hepatic progenitor cell proliferation and colony formation we began by analyzing the endogenous production of TGF-β1 by nonparenchymal cells. Over a 3 week period, there is a basal level of active TGF-β1 produced at a concentration that ranges between 20 and 47 pg/ml. (Fig. 1). This basal production is concurrent with HPC proliferation and colony formation. Surprisingly, when we established a dose–response curve of exogenous TGF-β1 in our HPC culture, the addition of TGF-β1 at concentrations as low as 0.005 ng/ml (5 pg/ml) inhibited HPC colony formation to approximately 50% of the control dishes. Two potential explanations exist for this dichotomous effect of TGF-β1 on HPC colony formation. First, it is possible that there is an additive effect between the exogenous TGF-β1 and the endogenously produced TGF-β1. Based on this theory, adding 0.005 ng/ml TGF-β1 would have only marginally increased the TGF-β1 concentration to ~50 pg/ml and we would not expect to see an impact on HPC colony formation. The results in Fig. 3 contradict this theory as there was a demonstrable negative effect on colony formation, even at this low concentration of exogenous TGF-β1. Another possibility is that the addition of TGF-β1 has an effect on the nonparenchymal cell population in addition to its effect on the HPC, thereby simulating an injured hepatic microenvironment. It is known that TGF-β1 is required for hepatic stellate cell activation [38], and the activation of the nonparenchymal cells could lead to an increased production of TGF-β as an inflammatory response thereby inhibiting HPC colony formation.

Growth inhibition by TGF-β in the liver is partly associated with enhanced apoptosis. TGF-β1 has been shown to induce apoptosis in primary hepatocyte cultures and hepatoma cells in vitro as well as in the regressing liver [18,39–41]. The apoptotic effect of TGF-β on proliferating hepatocyte populations has been extensively studied [18,22,36] with several authors demonstrating that a subset of fetal hepatocytes are resistant to TGF-β effects [26,27,42] and other authors have shown suppression of TGF-β mediated apoptosis via other growth factors (e.g., EGF) [28,39].

Demonstrating the differences between TGF-β1's effects on hepatic progenitor cells and other regenerating hepatocyte populations is a critical step to understanding how these cells can potentially be used as a clinical therapy. Exogenous TGF-β1 initially impacts the viability of progenitor cells by creating an environment where the cells do not survive and therefore cannot proliferate and form colonies. After the HPC have begun proliferating and initiate colony formation, nearly 100% of these cells appear resistant to TGF-β1's antiproliferative and pro-apoptotic effects. Based on the findings in these experiments it is likely that transplanting HPC into injured livers would lead to the same negative effect on the HPC engraftment and subsequent proliferation unless they were expanded ex vivo prior to their transplantation.

As we gain further insights into the hepatic stem/progenitor cell populations along with their interactions at the cell–cell, cell–matrix, and local cellular environment, we will be able to successfully design strategies for their use as a cell therapy. Understanding the mechanism of action of hepatic-related growth factors (e.g., TGF-β1) is a critical step as numerous liver-derived cellular populations produce this cytokine, especially in association with injury (e.g., cirrhosis, toxin exposure, and during liver regeneration) [38].

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