Elevated Interferon Gamma Signaling Contributes to Impaired Regeneration in the Aged Liver

Pallavi Singh,1,2 Triona Goode,2 Adam Dean,2 Samir S. Awad,3 and Gretchen J. Darlington2

1Columbia University, College of Physicians and Surgeons, New York, New York.
2Huffington Center on Aging and 3Department of Surgery, Baylor College of Medicine, Houston, Texas.

Address correspondence to Pallavi Singh, Ph.D., Department of Dermatology, Columbia University Medical Center, Russ Berrie Medical Pavilion, Room 307, 1150 St. Nicholas Avenue, New York, NY 10032. Email: pallavi.singh02@gmail.com

Our previous study on immune-related changes in the aged liver described immune cell infiltration and elevation of inflammation with age. Levels of interferon (IFN)-γ, a known cell cycle inhibitor, were elevated in the aging liver. Here, we determine the role played by IFN-γ in the delayed regenerative response observed in the aged livers. We observed elevated IFN signaling in both aged hepatocytes and regenerating livers post-partial hepatectomy. In vivo deletion of the major IFN-γ producers—the macrophages and the natural killer cells, leads to a reduction in the IFN-γ levels accompanied with the restoration of the DNA synthesis kinetics in the aged livers. Eighteen-month-old IFN-γ−/− mice livers, upon resection, exhibited an earlier entry into the cell cycle compared with age-matched controls. Thus, our study strongly suggests that an age-related elevation in inflammatory conditions in the liver often dubbed as “inflammaging” has a detrimental effect on the regenerative response.

Key Words: Aging—Inflammation—Gamma interferon—Liver regeneration—Partial hepatectomy.

Received January 30, 2011; Accepted May 2, 2011

Decision Editor: Rafael de Cabo, PhD

A DECLINE in the tissue regeneration potential with age is a common theme seen across several organ systems, including liver (1). In young rats, virtually all hepatocytes enter the cell cycle after 70% liver resection, whereas only one third do so in the aged liver (2). Delayed entry into the S phase is also observed, and the BrdU incorporation peak is shifted to later time points. Consistent with the delay in DNA synthesis, aged rats have lower activities of nucleotide metabolism enzymes—thymidylate synthetase and thymidine kinase post-partial hepatectomy (PH) (3,4). Similarly, after liver transplant in humans, the allografts from aged donors (>50 years) display a lower liver growth volume compared with the younger donors (<30 years) (5).

Several studies have shown an upregulation of the inflammatory response with age across various tissues, suggesting an age-related increase in the proinflammatory status (6,7). Differential gene expression profiling of young and aged livers using microarrays reveals an age-related upregulation of genes belonging to the immune response category with 40% of the genes classified as inflammation related (8,9). Elevated expression of acute phase genes, which are induced in liver during systemic inflammation along with the presence of microgranulomas, is also observed in the aging hepatic tissue (10). The total number of lymphocytes in the liver triplifies from the age of 7 weeks to 80 in mice (11). Interestingly, chronic inflammation induced by Concavalin A treatment or due to steatohepatitis causes a marked inhibition of liver growth after PH (12,13).

Conversely, immunosuppressive drugs such as FK506 and cyclosporine augment the regenerative response of the liver after resection surgery. Hepatocytes, cultured in serum drawn from immunosuppressed mice, display enhanced proliferation, indicating a detrimental effect of inflammatory factors on the proliferative response (14). Thus, a negative correlation seems to exist between chronic hepatic inflammation and the regenerative capacity of the liver.

Our previous study described an elevation in the transcript levels of various growth inhibitory inflammatory cytokines, such as transforming growth factor-β, interferon (IFN)-γ and interleukin (IL)-1 in the hepatic tissue (15). Another study describes an increase in IFN-γ production by splenic cells with advancing age (16). IFN-γ is known to be a potent inhibitor of the cell cycle, and treated hepatocytic cell lines exhibit growth arrest. IFN-γ inhibits the cell cycle through activation of cyclin–cdk complex inhibitor, p21. It binds to its receptor on the cell surface and causes the phosphorylation of the STAT1 protein, which further activates the IFN-specific transcription factor IRF1 (17). In fact, the IFN-γ-induced PH repression is abolished in Stat1−/− and Irf1−/− mice (18). IRF1 causes nuclear accumulation of p53 (19,20) and increased nitric oxide production via inducible nitric oxide synthase induction (21,22), which subsequently increases p21 transcription (23). Induced elevation of IFN-γ levels artificially by dI:dC leads to impaired liver regeneration. Gamma IFN knockout mice show enhanced regeneration as compared with controls (24). Cellular components
of the innate immunity—natural killer (NK) cells and macrophages are known to be robust producers of IFN-γ. An increase in the number of these cell types in the aged livers, as shown by our previous study, could augment IFN-γ levels both in the organ and in the circulation. We thus hypothesize that the increased presence of IFN-γ in the old livers can potentially lead to inhibition of the cell cycle and impaired regeneration after PH.

In our current study, we report elevated IFN-γ levels in the aged liver tissue compared with young. In addition, increased transcript levels of IFN-regulated genes are observed in the aged animals both before and after PH. We also tested whether the depletion of NK cells and macrophages leads to a decrease in the production of IFN-γ in the aged liver and whether the regeneration process in the liver improves in the absence of these cell types. Lower levels of IFN-γ were observed in NK cell and macrophage-depleted animals. BrdU incorporation rates were comparable to young at 38 hours, which is the DNA synthesis peak in young animals. We further tested the effect of the absence of IFN-γ on liver regeneration in the aged animals by studying the response of 18-month-old IFN-γ−/− mice to PH. Our study addresses the impact of inflammatory mediators, which have been shown to be upregulated with age, on the regenerative process in the liver and can help in understanding their effect on recovery after liver resection surgeries in aged patients.

METHODS

Mice

Aged CB6F1 mice were obtained from National Institute of Aging rodent colony and C57BL/6 and IFN-γ−/− mice from Jackson Laboratories. The animals were housed in groups of 3–5 animals of the same gender in a room with controlled photoperiod of 12-hour light–12-hour darkness (lights on from 07:00 to 19:00 hours) and a temperature of 22°C ± 2°C. Animals were given free access to water and pelleted diet (5010 rodent diet, LabDiet, PMI Nutrition International, Brentwood, MO). For tissue harvesting, the animals were anaesthetized with isofluorane followed by cervical dislocation; livers were collected, flash frozen in liquid nitrogen, and stored at −70°C. All procedures with animals were anaesthetized with isoflurane followed by cervical dislocation; livers were collected, flash frozen in liquid nitrogen, and stored at −70°C. All procedures with animals were carried out in accordance to the provided guidelines for laboratory animal welfare by the Association of Assessment and Accreditation of Laboratory Animal Care–approved Center of Comparative Medicine at Baylor College of Medicine, Houston, TX, under specific pathogen-free (SPF) conditions in microisolator cages.

Surgery and Antibody Injections

Mice anesthetized with avertin (1.2% avertin in 0.9% saline; 240 mg/kg body weight) underwent PH surgery as described (25). Median and lateral hepatic lobes were separately ligated with monofilament suture (cat# Z421H, Ehticon, Somerville, NJ) and excised through a 1-cm longitudinal abdominal incision, which was then closed in two layers. Excised tissue was flash frozen in liquid nitrogen as a 0-hour control as well as fixed in 10% formalin for histochromy. Animals were given BrdU injections (50 mg/kg body weight) before tissue harvesting at 32, 38, 40, and 48 hour after PH.

For NK cell depletion experiments, mice were injected intraperitoneally 24 hours before surgery with 100 μL of anti-asialo GM1 antibody (catalog # 986-10001 Waco chemicals, Richmond, VA) in 200 μL phosphate-buffered saline, whereas the control mice were given injections of normal rabbit serum (320 mg per animal). For macrophage depletion experiments, mice were injected intravenously 24 hour before surgery with 100 μL of 4.5 mg/mL solution of GdCl3 (catalog # G7532 Sigma) in 200 μL 0.9% saline, whereas the control mice were given injections of 300 μL of 0.9% saline.

Real-Time Polymerase Chain Reaction

Total RNA was extracted from frozen livers using the RNeasy purification kit (Qiagen) in accordance with the manufacturer’s protocol. DNase-treated total liver RNA was reverse transcribed using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA). Real-time polymerase chain reaction was performed using SYBR Green Master Mix and the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster city, CA). γ-tubulin and β-actin were used to normalize the RNA concentration in samples. Thermal cycling conditions consisted of an initial step at 95°C for 10 minutes to activate the Taq DNA polymerase and 40 cycles of sequential denaturation at 95°C for 15 seconds and annealing and extension at 60°C for 60 seconds. Data analysis was performed using the ABI Prism 7000 SDS Software (Applied Biosystems). The primers used are listed in the Supplementary Table 1. The real-time polymerase chain reaction analysis was performed according to the comparative C_{T} method (9). The p values reported for these changes refer to a two-tailed t test between the normalized C_{T} values in old versus young mice.

Immunohistochemistry

Livers, along with spleen from the same animal, were fixed in 10% buffered formalin, paraffin embedded, and sectioned to a thickness of (4 μm). The liver tissue sections were stained for BrdU incorporation using mouse antimes BrdU (Catalog # M0744, Dako cytometry) along with MOM kit (BMK-2202, Vector Laboratories) and counterstained with eosin. Sections were also stained for proliferating cell nuclear antigen (PCNA) and Histone3 phosphorylation levels using rabbit anti-mouse PCNA antibody (Catalog # sc7907, Santa Cruz) and rabbit anti-pH3 antibody (Catalog # 06-570, Upstate). Simultaneous staining with spleen was used as a positive control for proliferation.
markers. Immunohistochemistry imaging was carried out using Zeiss Axioskop 2 plus microscope, and images were processed using the AxioVision 3.1.2.1 image analysis software. The cells staining positive for each of the cell cycle markers were counted manually per field for multiple fields, and two-tailed t test for paired data sets was carried out using excel software to assess statistical significance.

Protein Isolation and Western Analysis

Nuclear, cytoplasmic, or whole-cell extracts were isolated according to the standard laboratory protocol. Briefly, liver tissue was homogenized using a Teflon mortar and pestle in buffer containing “COMPLETE” protease inhibitors (Roche) and 1mM NaF (phosphatase inhibitor). Western analysis of protein isolates was performed using either 50 or 100 µg of protein according to the standard laboratory protocol. Briefly, sodium dodecyl sulphate–polyacrylamide gels were transferred to polyvinylidene difluoride membrane, typically incubated overnight with primary antibody at 4°C (shaking) and then probed with secondary antibody for 1 hour at room temperature. Anti-Stat1 (# M22X, Santa Cruz) and anti-p-Stat1 (# 9171, cell signaling) are used to detect the stat1 levels in the liver.

Enzyme-Linked Immunosorbent Assay

Small pieces of frozen liver tissue were homogenized by a dounce homogenizer in phosphate-buffered saline. The tissue was spun at 15,000g for 10 minutes to pellet the tissue debris. The supernatant was used for cytokine analysis using the Bio-Plex Mouse Cytokine 18-Plex Panel (cat # 171-304000, Biorad). The assay kit contained microbead-coupled antibodies against murine cytokines. The Bio-Plex cytokine assay protocol was followed to carry out single-well multiplex against the cytokines using 50 µL of the sample along with the concentration standards. The contents of the microplate well were analyzed using the Bio-Plex array reader. The calculation of analyte concentration, standard deviation, and standard error was carried out using the Bio-Plex Manager software.

Microarray Analysis

See Supplementary Data.

RESULTS

Elevated Levels of IFN-γ Are Present in Old Animals Along With Higher IFN-γ Signaling in Hepatic Tissue Prior to and After PH

To determine the age-related difference in the message levels of IFN-γ, quantitative real-time polymerase chain reaction was carried out between complementary DNA obtained from young (6 months) and old (24 months) CB6F1 mice livers (n = 4). The levels of IFN-γ mRNA were found to be 13-fold (p < .05) higher in the old animals. Comparison of 24- versus 3-month old in another inbred strain C57Bl/6 (n = 5) also showed elevated message levels of IFN-γ with a fold change of 2.7 (p value < .001). Higher IFN-γ levels, as measured by multiplex enzyme-linked immunosorbent assay, were also observed, although due to high variance in the old animals, the differences did not reach statistical significance (p value = .19; Figure 1). Immunofluorescence staining for IFN-γ also confirmed its presence in lymphocytic foci in the aged liver. IFN-γ signals to both hepatocytes and nonparenchymal cells, which includes the lymphocytic population of the liver. To dissect and study the effect of IFN-γ on the hepatocytes, we carried out separation of the parenchymal fraction enriched in hepatocytes from the nonparenchymal cell fraction by perfusing the liver followed by differential centrifugation. Quantitative real-time polymerase chain reaction analysis was carried out for IFN-γ-regulated genes on the parenchymal fraction. The hepatocytes derived from livers of old mice showed a general elevation of message levels of these IFN-γ targets compared with the young mice with ifi1, icam1, pml, and ifngr1 displaying a statistically significant difference between young and old (Table 1).

Microarray gene expression data set comparing the transcriptional profiles of young and aged livers at 0.5, 1, 2, 4, 24, 38, and 48 hour after resection surgery was generated previously in our laboratory. To determine whether the IFN-regulated genes are differentially overexpressed in the aged livers after resection surgery, we selected two sets of genes from published studies exhibiting a greater than fourfold increase in response to IFN-γ(26,27) and compared the differential expression profile between young and old livers in our array data set at time points 0, 0.5, 1, 2, and 4 hour after PH (Figure 2A). The aged livers showed a general upregulation of the chosen genes compared with young livers with statistically significant higher expression (sign test; p value < .05) at time points 0.5, 1, 2, and 4 hour in Set A and 0.5 and 2 hour for Set B. In addition to the genes examined in the array data set, message levels of IFN-regulated genes measured by quantitative real-time polymerase chain reaction showed elevated expression in the aged livers with significant (p value < .05) upregulation in message levels of ifitm3 at 0 hours, ly6e at 1 hour, and irf7, ifi47, isgf3, and ly6e at 2 hours after PH (Figure 2B).

We further analyzed the downstream IFN-γ signaling pathway in the regenerating livers. Analysis of microarray gene profiling data showed that the expression levels of cell cycle inhibitor p21 were elevated in the aged livers at 24 hours accompanied with lower expression levels of cell cycle genes—cyclin D1, cdk4, E2F1, and PCNA involved in the G1/S transition phase, indicating a lag in cell cycle entry in old animals (Figure 3 and see Supplementary Table). The expression levels of Stat1 were significantly elevated at 0.5 and 2 hours, IRF1 at 1 hour, and inducible nitric oxide synthase at 2 hours after PH (p value < .05), indicating an
increased signaling through the pathway at the immediate early stage of regeneration (see Supplementary Figure 1). To further identify the genes that were significantly changing between young and aged animals and to determine their association with various pathways, we carried out network analysis using the software Ingenuity (Figure 3). Network associated with G1/S cell cycle transition identified several genes that were downregulated in the aged animals at 24 and 38 hours. Several of these genes such as replication protein A3, DNA topoisomerase, chek1, ribonucleotide reductase, high mobility group B2, replication factor C4 are for DNA synthesis, maintenance, and replication during S phase, explaining the delayed DNA synthesis in aged animals post-PH. Total protein levels of Stat1 and p-Stat1 were also measured by Western blot (Figure 4). The levels of total Stat1 were significantly elevated in the aged animals at time points 0, 1, and 2 hours after PH ($p < .05$). Phosphorylated Stat1 showed a significant elevation at 0 hours ($p < .05$), indicating increased IFN-γ signaling in the aged animals both prior to and post-PH. The activated Stat1 homodimer translocates to the nucleus and leads to transcriptional activation of IFN-specific transcription factors called IRFs or IFN regulatory factors (28). Thus, elevated IFN-γ and downstream-regulated gene message levels in the aged liver indicate an overall increased responsiveness to the IFN-γ signaling pathway.

Depletion of NK Cells and Macrophages Leads to Improved Regeneration in Aged Liver Accompanied With a Decrease in IFN-γ Levels

Our previous study showed increased infiltration of the aged liver with immune cells, such as macrophages, NK cells, T-cells, B-cells, and neutrophils (15). Resident macrophage (Kupffer cells) and NK (pit cell) population of the liver plays an important role in regulation of the immune functions of the liver. Kupffer cells are a part of the reticuloendothelial system, which aid in the clearance of foreign antigens from
the portal circulation, whereas NK cells carry out tumor surveillance functions. Both these cell types are potent inducers of inflammation and secrete large amounts of IFN-γ when stimulated. To test whether they play an inhibitory role in the hepatic regeneration process in the old animals, we depleted the liver of NK cells or macrophages. CB6F1 mice, aged 18 months, were injected 24 hours prior to PH with either anti-asialoGM1 (AsGM1) antibody, which depletes the liver of NK cells, or normal rabbit serum as a control. Depletion of NK cells in the old animals was assessed by measuring the transcript levels of NK cell–specific markers using quantitative real-time polymerase chain reaction. Antibody administration led to a significant decrease in the expression of NK cell–specific transcript levels of nkr-p1c, nkr-p1a, and cd94 and a 2.8-fold decrease in the message levels of IFN-γ compared with the control animals (Figure 5).

The macrophage-depleted animals showed higher levels of BrdU compared with the control animals. Interestingly, comparison of the levels of BrdU incorporation in NK- and macrophage-depleted aged animals with young mice (6 months) showed no significant difference (Figure 5). These data suggest that both NK cells and macrophages impede liver regeneration in aged livers. The concomitant decrease in the levels of IFN-γ with the depletion of macrophages and NK cells makes this cytokine a strong candidate for the growth inhibition observed in the aged livers, yet we cannot exclude the possibility that other cytokines/cell products could also play a similar role in aged animals.

Liver Regeneration in Aged IFN-γ−/− Mice

IFN-γ signaling, known to arrest growth by induction of cell cycle inhibitors, is elevated both before and after PH in the aged livers. Thus, we further tested whether the absence of IFN-γ in the aged liver has a positive effect on the regenerative process. PH was carried out on 18-month old IFN-γ−/− mice and C57BL/6 age-matched background controls. In general, the liver tumor incidence in these mice was higher, and the mice had very high mortality rates post-hepatectomy. PCNA is expressed during early G1 and S phase, acts as the processivity factor for polymerase delta during DNA synthesis, and is commonly used as a histological marker for actively dividing cells. The percentage of PCNA positive cells in the IFN-γ−/− mice were significantly greater than in the control group.
those of the wild-type (WT) animals at time points 32 and 40 hours, whereas at 48 hours, the levels were almost similar, suggesting that IFN-γ−/− mice enter the cell cycle earlier than the control mice. The average BrdU incorporation levels were higher in IFN-γ−/− animals at early time points 32 and 40 hours compared with WT controls, although the differences were not significant (Figure 6). Hence the absence of IFN-γ seems to have a positive effect on the cell cycle kinetics of the aged liver.

**DISCUSSION**

Few organs in the body can compare with the remarkable regenerative potential of the liver, which, being the metabolic and xenobiotic detoxification hub of the body, produces and neutralizes a large quantity of free radicals, frequently incurring heavy chemical insults. This ability for regrowth seems to be compromised as several age-related changes occur in the liver, which have subsequent impact on the regenerative potential. It has been observed that a cluster of symptoms, including high body mass index, elevated blood pressure, high triglyceride and cholesterol levels, and insulin resistance collectively falling under the metabolic syndrome category, become more prevalent in elderly persons (29,30). Age-related metabolic syndrome is also associated with a proinflammatory state with appearance of inflammatory markers such as c-reactive protein, fibrinogen, PAI-1 and homocysteine levels (29,31,32) and shown to be a significant factor in the development of non-
Figure 3. Changes in the G1/S transition between young and aged mice. (A) Figure shows the expression levels of cell cycle genes cyclinD1, cdk4, E2F1, and PCNA at time points 24, 38, and 48 hours after partial hepatectomy (PH) in aged and young mice ($N = 3$). * denotes $p$ value < .05. (B) The G1/S transition network describes the old/young fold change in the expression levels of the cell cycle genes in the regenerating liver at 24 hours post-PH liver. The fold changes are colored on a scale ranging from green denoting relative downregulation of genes to red indicating a relative upregulation. Solid lines denote direct physical interactions, whereas dotted lines represent indirect regulatory interactions between the genes. The genes in the network exhibit a fold change regulation of 1.5 times or more and a $p$ value of interaction < .01.
alcoholic fatty liver disease, a condition with increased fat deposition in the hepatocytes. A study involving Japanese patients found that the incidence of development of nonalcoholic fatty liver disease was much higher in the patients diagnosed with metabolic syndrome prior to the study (33). In the same vein, a study by Einstein and colleagues (34) showed that aging confers susceptibility to free fatty acid–induced insulin resistance and release of proinflammatory cytokines by adipose tissue, which can further contribute to liver pathology. Antioxidants, certain metabolism-modulating compounds, and antiinflammatory drugs show a positive effect on the general health span (35). Dietary resveratrol supplementation has been shown to reverse hepatosteatosis in Wrn mice model of accelerated aging (36). Fat accumulation and subsequent development of steatohepatitis might have an adverse effect on the regenerative capacity of the liver. Mice fed a western diet rich in saturated fatty acids and cholesterol prior to CCl$_4$-induced liver injury show blunted regrowth (37). Mice maintained on a high-fat diet as well as leptin receptor–deficient obesity-prone mice develop steatosis and are more susceptible to hepatic damage and regenerative defects when subjected to PH (38,39).

As the liver ages, there are several changes in the structure as well as the DNA content of the hepatocytes. Polyploidy, which is either an increase in the number of nuclei or the number of genomes within a single nucleus, increases with age and corresponds inversely to the senescent age of the organism (18–28 months) (40–42). Binucleation and polyploidization are irreversible differentiation events, which have been shown to negatively affect the growth potential of the liver. The diploid hepatocytes show higher proliferative activity than polyploid cells after PH or treatment with mitogenic compounds (43,44). Reduced telomere length is one of the critical factors contributing to cellular aging and replicative senescence (45,46). Interestingly, hepatocytes exhibit one of the highest telomere attrition rates, which could play a detrimental role in the ability to regenerate (47). Assessment in patients suffering from cirrhosis and chronic hepatitis also reveals a significantly lower telomeric length in comparison with age-matched patients, indicating a negative relationship of aging and/or inflammatory conditions with telomere length (48,49). Interestingly, administration of growth hormone as well as exposure to circulating factors from young via heterochronic parabiosis restores the regenerative deficits in the aged animals (50,51).

Aging is accompanied by an increase in the inflammatory status of the organism dubbed as inflammaging (7) and is associated with the remodeling of the immune system. The numbers of hepatic natural killer T-cells as well as the γδ T-cells go up with age (52,53). An elevation of T-cell receptor intermediate T-cells and mononuclear cells numbers is also observed in the liver (54). Unpublished microarray gene expression data from our laboratory revealed a consistent
upregulation of inflammation-related genes in the aged liver before and after PH in CB6F1 mice. We further demonstrated the presence of a proinflammatory microenvironment in the aged liver associated with immune infiltrates comprising of macrophages, T-cells, B-cells, NK cells, and neutrophils, which exhibit properties of lymphoid neogenesis (15). The liver exhibits increased injury when these inflammatory conditions are recapitulated by LPS injection lipopolysaccharide (LPS) (55). Hence, there is a general increase in the inflammatory conditions with age, conferring susceptibility to hepatic injury.

Several lines of evidence suggest that innate immunity plays an important role in the coordination of the regeneration process. As soon as the liver undergoes resection, the flux of LPS or the bacterial antigens in the remnant liver increases, leading to increased activation of toll-like receptor pathway, which seems to act as one of the triggers for regeneration. C3H/HeJ mice defective in response to LPS as well as Gram-negative bacteria–free mice display a delayed liver regeneration response (56). Toll-like receptors bind the LPS in circulation and through signaling via MyD88 induce various proinflammatory cytokines by Kupffer cells. The MyD88−/− mice exhibit a marked delay in the expression of immediate early genes as well as a delayed regeneration response post-PH (57). LPS activation of hepatic nonparenchymal cell population leads to an increased secretion of TNF-α and IL-6, which in turn induce the acute phase response in the liver and activation of immune cells. During liver regeneration, these cytokines signal through their respective receptors on the hepatocytes and prepare them for stimulation by growth factors, an event known as priming. The mice deficient in IL-6 (IL-6−/−) (58) and TNF-α receptor–deficient mice show delayed regeneration response and increased necrosis. Components of the complement cascade, which help in antigen clearance and mediation of inflammation, are also essential for repair and regeneration. Mice deficient in complement components C3 and/or C5 exhibit parenchymal damage and impaired regeneration (59). Microarray study on the post-PH tissue from rats has yielded close to 85 genes whose levels change during liver regeneration and which are classified functioning in innate immunity pathway, suggesting its significant role in liver regeneration (60).
Although innate immunity is an integral part of regeneration, the presence of chronic inflammatory conditions is detrimental to the regenerative process. Conditions of liver inflammation such as artificially induced hepatitis by administration of concavalin A (12) and inflammation associated with severe steatosis (13) both show a decrease in the proliferation and reduced levels of cell cycle genes in the mice. Inflammatory conditions in liver are associated with increased levels of proinflammatory cytokines, such as IL-6, TNF-α, IFN-γ, and transforming growth factor-β. Although IL-6 and TNF-α have been shown in many studies to play a central role in the initiation of the priming events, preexisting elevated levels of both IL-6 and TNF-α can be detrimental to regeneration. Mice hyperstimulated by injection of IL-6 exhibit a strong activation of stat3 accompanied with inhibition of hepatocyte proliferation as measured by BrdU staining and Cyclin A and E expression (61). In ob/ob mice, which exhibit chronically elevated IL-6 and p-stat3 levels, there is an elevated accumulation of p-stat3 in the nucleus after liver resection, leading to induction of the cell cycle inhibitor p21 and cell cycle arrest (62). Similarly, in a model of overexpression of TNF-α (TIMP3−/−), a heightened hepatic lymphocyte infiltration, necrosis, and inhibition of regeneration was observed after PH (63). The stop signal to cease proliferation is initiated in part by the proinflammatory cytokine transforming growth factor-β, which is a potent inhibitor of hepatocyte growth. Deletion of transforming growth factor-β from liver tissue using neutralizing antibodies or genetic knockout also leads to a better regenerative response post-PH (64,65). Thus, chronic inflammation seems to have an adverse effect on the regenerative process despite an important role of innate immunity in the initiation of regeneration. In this light, the therapeutic role of caloric restriction/methionine restriction and the insulin/insulin-like growth factor pathway could be important as these mice models show a lower rate of aging, accompanied by lower inflammation and better hepatic response to chemical insult and PH (66–68).

Macrophages are known to be central players in the slow ramping up of inflammation with advancing age (7). Kupffer cells form cell clusters in the parenchyma compared with the younger liver, reminiscent of the inflammatory granulomatous disease (15), and exhibit elevated production of proinflammatory cytokines IL-6 and TNF-α. A 70% knockdown of macrophage numbers using gadolinium chloride leads to improvement of regeneration due to reduction of production IL-10, which inhibits TNF-α (69). Numbers as well as activity of NK and natural killer T-cells are also elevated with age in the liver (70). These cells have been implicated in hepatic injury during liver regeneration along with increased production of IFN-γ (71,72). Administration of poly dI:dC, which elicits IFN-γ production or direct IFN-γ treatment causes repression of proliferation and increases the expression of several antiproliferative proteins, including STAT1, IRF1, and p21 in mice livers after PH (24). The impairment of liver regeneration in poly dI:dC-treated animals is abolished in stat1−/− and IRF−/− mice implicating their role in downstream signaling (18).

We tested the effect of absence of IFN-γ on liver regeneration in the aged animals. PH experiments carried out on aged IFN-γ−/− mice showed higher levels of the proliferative marker PCNA earlier in the cell cycle compared with the WT controls. The levels of BrdU staining, although not statistically significant, were also higher in the IFN-γ−/− mice at 32 hours after PH in suggesting an overall earlier entry into the cell cycle compared with WT mice.

Liver resection is commonly and successfully used for the treatment of malignant liver tumors with high long-term survival rates. In elderly patients, the mortality rates associated with liver resection are higher (11%) compared with a younger population (2%–4%), and in cases of extensive resection, these rates increase to 30%. Interestingly, when the elderly patients suffering with cirrhosis are excluded from the data set, the mortality rates drop to levels comparable to the younger population (73), suggesting that cirrhotic patients have either higher inflammation or fewer healthy hepatocytes. Chronic liver disease has been associated with higher risk of liver failure following PH due to presence of
extensive cirrhosis and ongoing inflammatory activity (74,75). Nonalcoholic fatty liver disease and accompanying steatohepatitis have been found to enhance the risk in major liver resections including postoperative liver failure (76) and can be detrimental to graft function in liver transplantation (77,78). Fulminant hepatic failure is the development of massive acute liver injury with rapid clinical deterioration in a previously healthy patient, and in the elderly patients, the condition is more acute with greater terminal complications compared with young patients (79). Induction of IFN-γ and increased NK cell activity in balb/c mice using the drug FS-112 causes fulminant hepatic failure and an impaired regeneration response (80). In human cases of fulminant hepatic failure, the serum levels of IFN-γ were elevated along with increased numbers of CD8+ IFN-gamma+ T lymphocytes (81).

Our study indicates a similar deleterious effect of innate immunity and IFN-γ in aged mice, wherein the deletion of the inflammatory mediators elicits a better liver proliferative response after liver resection. Thus, studies addressing the impact of age-related increase in inflammatory conditions specifically IFN-γ on regenerative process in the liver can help in understanding their effect on recovery after liver surgeries in elderly patients.

Funding
This work was supported by the National Institutes of Health (PO1 AG20752 and AG028865); the Texas Medical Center Digestive Disease Center (DK56338 and DK53045); and the Ellison Medical Foundation.

Supplementary Material
Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

Acknowledgments
We thank Dr. Milton Finegold for his expertise in the histological analysis of the liver samples. We also thank the Digestive Disease Center, Baylor College of Medicine, for help with immunohistochemical analysis. P.S.—experimental design, animal surgeries, microarray analysis, and data analysis; T.G.—animal surgeries, early time points and microarray analysis; S.S.A.—rodent survival surgery techniques; A.D.—technical support; and G.J.D.—experimental design and data analysis.

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