Liver regeneration – The best kept secret. A model of tissue injury response

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ABSTRACT

Liver regeneration (LR) is one of the most amazing tissue injury response. Given its therapeutic significance has been deeply studied in the last decades.

LR is an extraordinary complex process, strictly regulated, which accomplishes the characteristics of the most evolutionary biologic systems (robustness) and explains the difficulties of reshaping it with therapeutic goals.

TH reproduces the physiological tissue damage response pattern, with a first phase of priming of the hepatocytes –cell-cycle transition G0-G1–, and a second phase of proliferation –cell-cycle S/M phases– which ends with the liver mass recovering.

This process has been related with the tissue injury response regulators as: complement system, platelets, inflammatory cytokines (TNF-α, IL-1β, IL-6), growth factors (HGF, EGF, VGF) and anti-inflammatory factors (IL-10, TGF-β).

Given its complexity and strict regulation, illustrates the unique alternative to liver failure is liver transplantation.

The recent induced pluripotential cells (iPS) description and the mesenchymal stem cell (CD133 +) plastic capability have aroused new prospects in the cellular therapy field. Those works have assured the cooperation between mesenchymal and epithelial cells.

Herein, we review the physiologic mechanisms of liver regeneration.

Key words: Liver regeneration. Sterile inflammatory response. Cell-cycle. p53. Hyperproliferative stress response. Induced pluripotential cells (iPS).

ABBREVIATIONS

AP-1: Activating protein-1.
Abs: Biliary acids.
ATP: Adenosine 5-triphosphate.
AR: Amphiregulin.
ARNm: Messenger ribonucleic acid.
Cdc25b: Protein phosphatases that dephosphorylates Cdk.
Cdk: Cyclin-dependent kinase.
CD117: Hematopoyetic progenitor cells.
CD133: Endothelial progenitor.
Ckls: Cdk-cyclin complex inhibitor.
Cl4C: Carbon tetrachloride.
C-met: HGF receptor.
CT-1: Cardiotrophin-1.
C3a: Proteolitic fragment “a” from C3 complement protein.
C3b: Proteolitic fragment “b” from C3 complement protein.
C5a: Proteolitic fragment “a” from C5 complement protein.
Check-point R: Cell-cycle “restriction” check-point.
DAMP: Damage-associated molecular pattern.
DNA: Deoxyribonucleic acid.
EGF: Epidermal growth factor.
EGFR: Epidermal growth factor receptor.
EM: Extracellular matrix.
FGF: Fibroblast growth factors.
FXR: Farnesoid X receptor.
gp 130: Glycoprotein 130.
GSK3b: Glycogen synthase kinase 3b.
G0: Quiescent or resting cell state.
G1: GAP 1 phase. Period within cell-cycle following mitosis.
G2: GAP 2 phase. Period within cell-cycle between the end of DNA synthesis and the beginning of M phase.
HB-EGF: Heparin-bound epidermal growth factor.

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e-mail: fjacien@unav.es
HGF: Hepatocyte growth factor.
HIF-1α: Hypoxia induced transcription factor-1α.
HMGB1: High mobility group Box 1.
IkBβ: Cytoplasmic NF-κB inhibitor.
IL: Interleukin.
IL-1β: Interleukin 1 β.
IL-6: Interleukin 6.
IL-18: Interleukin 18.
IL-6R/gp130: IL-6 glycoprotein 130 receptor.
iPS: Induced pluripotent stem cells.
JAK: Tyrosine kinase Janus or Janus kinase.
KO: Knout-out.
LPS: Lipopolysaccharide protein.
LR: Liver regeneration.
M phase: Cell-cycle mitosis phase.
MAPK: Mitogen-activated protein kinase.
Myc: Myc oncoprotein.
NF-κB: Nuclear factor Kappa β.
NK: Natural killer.
NKT: Natural killer T cell.
NLRP: NOD-like receptors.
NOD: Nucleotide digomerization domain – containing protein.
PAF: Platelet activator factor.
PAMP: Pathogen associated molecular patterns.
PDGF: Platelet derived growth factor.
PHx: Partial hepatectomy.
ROS: Oxygen free radicals.
RPK: Receptor tyrosine kinases.
RTK: Receptor tyrosine kinases.
S phase: Cell-cycle DNA replication: synthesis phase.
SOCS: Suppression protein of cytokine signaling.
SOCS-3: Cytokine signaling suppression.
STAT3: Signal transducer and activator of transcription.
TFG: Transforming growth factor

TNF-α: Tumor necrosis factor-α.
TNF-R: Tumor necrosis factor receptor.
TPM1: Tryptophan hydrolasa.
VEGF: Endothelial-vascular growth factor.

“Nature adapts the organ to the function and not the function to the organ”
Aristotle, on The parts of the animals

BACKGROUND

Liver regeneration (LR), is one of the most enigmatic and fascinating phenomenon of the animal scale. The rapid volume and function recovery following a major liver resection (> 70 %) or liver injury and its strict regulation at the initiation and termination periods is an exclusive characteristic of the liver (1-9).

In addition, LR is the scientific background of several clinical procedures as the extended liver resections (> 70 % of the liver parenchyma or five segments), split liver transplantation, liver donor procedure, two-stage hepatectomies, isolated portal vein embolization or associated with “in situ” liver transection, the artificial liver support in acute hepatic failure and the clinical application of cellular therapy as well (10-21) (Fig. 1).

Albeit the liver regenerative ability was described in the punishment inflicted to Prometheus by Zeus, the first scientific description was reported in 1879 (22-24). This ability represents one of the most extraordinary physiological response in maintaining the internal homeostasis. Besides the myriad of metabolic functions, the liver is the major immune organ and the first natural filter for microorganisms (bacteria, virus) and xenobiotics originated in the intestines (25-28). In the last 50 years has been an extraordinary interest in knowing the underlying mechanisms of the LR, and the mentioned clinical applications (4,7,29-31).

In the present article we review the physiologic basis underlying LR and its relations with clinical conditions.

![Fig. 1. A. Intraoperative view of the remnant liver (segments 1, 2, 3, 4) following right hepatectomy in a living donor procedure. B. Radiologic imaging of the regenerating remnant left lobe (segments 1, 2, 3, and 4) 1.5 months post-hepatectomy after right hepatectomy in a living donor procedure (courtesy of Dr. A. Benito. Radiology Department. Clinica Universidad de Navarra).]
LIVER REGENERATION CHARACTERISTICS

In basal conditions the hepatocytes remain in quiescent state (phase G0 of the cell cycle), but retain the ability to reinitiate the cell cycle with occasion of tissue damage, tissue loss or exogen stimulus (growth factors, mitogens) (32-35). LR has been studied in diverse experimental models; from cellular cultures to “in vivo” induced liver injury by toxics (Cl4C, galactosamine, thioacetamide), bacterial particles (LPS), virus and surgical models as partial hepatectomy (PHx) or segmental liver transplantation. Knock-out (KO) and knock-in animals with a genetic over-expression or a specific gen deletion have been used too (2,5,36-38). Others authors have investigated the gen expression profile with microarray techniques (39-43). Some of the mentioned models are summarized in table I.

From the above mentioned models highlight those in which the liver volume, function and survival were evaluated. Yet the KO models have been crucial for the signal pathway identification, their assessments have been difficult because LR pleiomorphic feature (40).

The 70 % PHx procedure in rodents and in big animals as well, is the most used, because represents the major regeneration stimulus and elicits the synchronic and homogeneous response of the remnant liver (36-38). This model has allowed to compare the regeneration between control animals (wild type) with the genetic modified on signaling molecules, receptors and cell cycle regulators (2,5,44).

The liver mass recovery following a PHx or after an acute injury is achieved by a compensatory hyperplasia in contrast with a true epimorphic regeneration of the lost tissue; that takes place in the inferior vertebrate-Zebra fish, Salamander or amphibians which are able to renew the removed limbs or other anatomical structures-major appendages, inferior jaw, portions of intestine, cardiac ventricle along the life time (45-49). These animals regenerate the lost tissue from the “blastema” formed by the transdifferentiaton of adult cells into mesenchymal cells (45,47,48).

The liver regeneration characteristics can be summarized in the following:

1. It is a phenomenon present in all vertebrates from Zebra fish to the human; in whom common mechanisms are involved: Thrombin activation, innate immune system participation, ploidy and aneuploidy development, tissue remodeling and the strict regulation of liver volume at the end of the process known as “hepatostat” regulation (34,46,50,51).

2. LR represents a strict regulated response, according with the pattern depicted in figure 2. The hepatocyte duplication, the mesenchymal cells duplication sequences, the morphogenesis and volume reached at the end of the process are genetically regulated. It is phenomenon different form embryogenesis or wound healing although share common signaling and transcription pathways (4,49,52).

<table>
<thead>
<tr>
<th>Targeted gen</th>
<th>Brief description of findings</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleucina-6 (IL-6)</td>
<td>Hepatic necrosis. Severe impaired LR. Role of IL-6 in G0</td>
<td>225</td>
</tr>
<tr>
<td>STAT3</td>
<td>DNA synthesis decreasing. Alterations in cell-cycle regulation</td>
<td>192, 195</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Stimulates G0 to G1 transition of cell-cycle</td>
<td>44, 72, 118</td>
</tr>
<tr>
<td>Gp 130</td>
<td>LR impairment following LPS administration</td>
<td>72, 196</td>
</tr>
<tr>
<td>IKK2</td>
<td>Deletion of IKK2 inhibitor, promotes innate response and hepatocyte proliferation</td>
<td>196</td>
</tr>
<tr>
<td>Complement C3a/C5a</td>
<td>Complement fractions are essential in early phases</td>
<td>170, 199</td>
</tr>
<tr>
<td>Farnesoid X receptor (FXR)</td>
<td>Mice deficient in FXR suffer a LR delay and increasing mortality</td>
<td>180</td>
</tr>
<tr>
<td>C-met</td>
<td>HGF and MET receptor are vital in cell-cycle entry following PHx</td>
<td>197, 271</td>
</tr>
<tr>
<td>FosM1b</td>
<td>Overexpression stimulates hepatocytes mitosis entry</td>
<td>247</td>
</tr>
<tr>
<td>EGF</td>
<td>Mortality increasing after Phx. KO mice suffer a delay in hepatocyte division</td>
<td>274</td>
</tr>
<tr>
<td>Inhibitor ILK</td>
<td>KO mice develop increasing proliferation and hepatomegaly</td>
<td>299, 300</td>
</tr>
<tr>
<td>TGF-α</td>
<td>Overexpression induces hepatomegaly. DNA synthesis augmentation</td>
<td>280, 281</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Transgenic mice suffer a delay in cellular proliferation</td>
<td>289, 290</td>
</tr>
<tr>
<td>TGF-β R2</td>
<td>KO mice show an early DNA synthesis</td>
<td>291</td>
</tr>
<tr>
<td>p53</td>
<td>KO mice develop high ploidy. p53 regulates the three phases of LR</td>
<td>117</td>
</tr>
</tbody>
</table>
3. It is a very rapid and explosive process which initiates in seconds and ends in few days –depending on the age and species– with the recovering of the hepatic volume and involves the signaling mechanisms –autocrine, paracrine, endocrine– of cell proliferation, morphogenesis, liver zonation and metabolism regulation genes (1,2,4,5,37,44,53). In recent years, diverse signaling and transcription pathways have been described, many of them related with the physiologic liver response to cell injury. Herein we shall describe how LR process reproduces the tissue damage response pattern (54-56).

LR begins with the recognition of the pathogen-associated molecular patterns (PAMPs) and the damage-associated molecular patterns (DAMPs); triggering the natural immune response-C3a and C5a complement fraction activation, TNF-α, TNF-α secretion, IL-6, IL-1β, IL-18 synthesis which induce the hepatocytes to proliferate. The transition from the resting G0 state to the G1 phase of the cell cycle (priming phase), comes about during the first 4 hours after liver resection (57-67).

An increasing in the preformed cytosolic preformed factors (TFs) –signal transducer and activator of transcription (STAT3), nuclear-Kappa β factor (NFKB) and activating protein 1 (AP-1)– have been described in the first 30 min post-hepatectomy as consequence of the signaling transductions induced by cytokines bindings with their receptors. TFs trigger the protein synthesis and gene expression required for initiating the cell cycle (known as priming phase). DNA synthesis increasing (phase S) has been reported during the 24 and 36 hours post-hepatectomy in the rat and in mouse respectively. The hepatocytes enter into mitosis at 48 hours post-PHx, following by kupffer...
cells. The stellate and biliary epithelial cells enter into S phase 48 hours post-hepatectomy and proliferate in slow pattern. Endothelial cells proliferate in the third day showing a peak at the fifth post-PHx day (3,4,42,68,71-73).

Additionally, a hepatocyte population return to the resting state (G0) at 72 hours post-hepatectomy meanwhile other reinitiate the cell division until reaching the original liver size (4,6,72). It has been postulated, that the second proliferative wave is due to growth factors secreted by the own hepatocytes and to the additive effect of co-mitogens such as norepinephrine, insulin, somatostatin and glucagon (1,2,4).

Miyakota et al. (52) has recently described that hepatocytes duplicate their size and increase the hepatocyte number by 1,5 fold, following PHx. These authors have reported an increase in the hepatocyte size by 1,6 and 0,7 divisions per cell suggesting that LR underlies two different processes: Hypertrophy and proliferation. The same authors observed in 30 % liver resection model the liver remnant increasing size was due to a significant 1,5-fold increase in the hepatocyte size without undergoing cellular division (52).

From the morphological point of view, several cluster of hepatocytes (10-14 mononuclear and binuclear ploidy cells –tetraploid (4n), octaploid (8n), 16n…) were observed shortly after PHx. A replication of the stellate and sinusoidal cells, increasing the size of liver lobules, was observed two-four post-hepatectomy days (35,74,75).

Ding et al. (76) has described the secretion of angiocrine factors and vascular neo-formation. The relative hypoxia of the residual liver due to overactive hepatic arterial buffer response, stimulates the hypoxia-induced factor 1 alpha (HIF-1α) –within the first 12-48 post-hepatectomy hours– which activates the vascular-endothelial growth factor (VEGF), the fibroblast-growth factor (FGF) and the inducible nitric oxide (NO) synthase (77-81). Proliferation of hepatocytes proceeds from the periportal to pericentral areas of the liver lobule: Once the original size has been restored an apoptotic remodeling and zonalizing process takes place (42,44,72,82,83).

5. In contrast to other solid organs, as skin, or the intestines, the restoration of liver mass is achieved by the mature hepatocytes and mesenchymal cells proliferation neither by pluripotent stem cell differentiation or oval cells; although its participation has been described whenever hepatocyte division is impaired or abolished as occurs in chronic liver diseases (1,2,3,5,42,44,72,84-87).

With occasion of the oval cell-hepatocyte precursor description and the plasticity of the hematopoietic cells, endothelial progenitor cells (CD133, CD117) and the induced pluripotent cell (iPS) described by the 2012 Nobel Prize Shinya Yamanaka (88-90); an explosion of articles regarding cellular therapy in liver diseases have been published (91,92). Given the enormous number of papers, we refer to excellent reviews (93-97).

Despite the aroused expected outcomes, the results have shown limited efficacy so far. Even trials performed with isolated adult hepatocytes in inborn errors of metabolism the results are very limited. In the majority of cases only a temporarily improvement was achieved (20,98). Preliminary clinical trials using bone marrow growing factors (G-CSF) in acute-on-chronic liver failure, and bone marrow progenitor cells (CD133) in chronic disease have recently published (96-99). Clinical results as happened with other solid organs (heart, pancreas, intestine) suggest that tissue regeneration is indeed a complex process which depends of the cooperation between different cellular lineages (87,100-102).

6. The liver regenerative capacity is almost unlimited. Seven consecutive 50% partial hepatectomies, without developing liver failure or preventing the regenerative response has been described in the rat (2-4). In a model of homozygote tyrosinemia (FAH –/–), the metabolic disorder was reverted after syngeneic hepatocyte transplantation. When the grafted hepatocytes were again isolated and transplanted they were able to reverse the enzymatic deficit in a FAH –/– second generation, and successively until 10 generations! It has been estimated that mouse hepatocytes could undergo up to 69 divisions, equivalent to generate 50 livers (103,104).

7. Another intriguing enigma of LR is the tight relationship between the hepatic volume and body surface; known as Liver Index (liver weight/body weight x 100 = 2.5 %); called as “hepatostat” regulation. In clinical and in experimental liver transplantation field, it is well known the adaptation of the donor liver size to the recipient body surface; small grafts in big recipients and vice versa. This phenomenon has even been reported in the baboon xenograft to the human (4,105-107).

Albeit most of the cellular changes throughout LR have been related with the cell nuclear changes; during cell division the organelles have to duplicate too. The regulation between cell growth and chromosome duplication is poorly understood (69,108-110). The majority of growth factors (epidermal growth factor –EGF–, hepatocyte growth factor –HGF–, insulin growth factor –IGF–) besides to their mitogenic effect, they increase the growth rate and promote cell survival by inhibiting apoptosis (5,69,108).
In inferior vertebrates and mammals is common to observe ploidy hepatocytes, tetraploid (4n) or octaploid (8n), mononucleated or binucleated (2x2n, 2x4n, 2x8n) as consequence of incomplete cytokinesis (Figs. 3 and 4). Ploidy degree differs across many species. In rats, 80% of hepatocytes are polyploid and aneuploid, 60% in mouse and 30% in human being (75,111). Ploydia’s first consequence is the hepatocyte increasing size (2,112,113). It has been pointed out that cellular size relies upon the DNA nuclear amount. Likely an increase in the chromosome number correlates with an increase in cell size (114-116).

Following a hepatectomy in rodents a 20% to 5% decreasing in binucleated hepatocytes and an increasing in tetraploid-4n- and octaploid-8n-, and even up to 16n hepatocytes have been described at 72 hrs post-HPx; showing that HPx represents and intense stimulus for DNA replication before cytokinesis (2,112,116,117). In those animals in which chromosome separation was inhibited, the recovery of liver mass following HPx took place by the hepatocyte polyploidy response, 18n, 32n, and higher degree (118,119).

This phenomenon coined as endorreduplication gives rise to chromosome replicating sequences without cellular division, generating cells with several genome copies, enhancing gene expression (120). Duncan et al. (35,74,111) has recently described the ability of hepatocytes to develop polyploidy and its reversion to aneuploidy state as an adapting response to xenobiotics or dietetic stimulus. It is remarkable that the liver function remained stable throughout the process, revealing the functional equivalence between 2n, 4n, and 8n hepatocytes (2,112).

Fig. 3. Microscopic imaging of a liver section showing diploid and tetraploid hepatocytes in an 8 weeks old mouse after 30 hours of fasting (H&E, courtesy of M. Bustos. CIMA. University of Navarra).

Fig. 4. Microscopic imaging showing diploid and hepatocytes in a 8 weeks old mouse after 30 hours of fasting (β-catenin staining, courtesy of M. Bustos. CIMA. University of Navarra).
Kurinna et al. (117) has recently published the p53 control role in quiescent hepatocyte as well as during regeneration (7 days). The authors described that KO mouse for p53 (p53 –/–), show a higher ploidy degree than control mouse (wild type, p53 +/+), and that they initiate earlier the cellular division, develop an increasing nuclei and cytoplasm size, and a higher degree of ploidy contrasting with p53 +/+ (wild type) after 70 % heptectomy. p53 lacking impairs ploidy reversion, confirming that regeneration in p53 –/– rodents likely is based in cell size augmentation as well. The mentioned authors found more errors in chromosomal integrity in p53 –/– mouse (multipolar spindle, lagging chromosomes) than in control animals (p53 +/+); yet the recovery of liver mass at 7 days post-hepatectomy was equivalent (52,117,121,122).

8. Several authors have pointed out that a more intense liver damage (extreme liver resections > 70 %, living donors transplantation with small grafts < 30 %, submassive hepatic necrosis), induces a stronger proliferative liver response (52,113-116). In liver donor transplantation setting using smaller grafts (≤30 % of standard liver volume) an accelerating in graft regeneration has been described (123,124). This pattern is similar to the classical physiological stress response or to tissue damage response as well (32,125-132).

9. Despite its amazing metabolic complexity, the liver keeps the homeostatic functions-albumin, coagulation factors synthesis, xenobiotic clearance, etc., throughout the regeneration process. A selective enhancing of proliferative genes and a temporarily silencing of carbohydrates metabolic genes were reported in the first 40 post-hepatectomy hours (42,72). Studies with light microscopy have revealed perportal changes of the classical lobule structure, 24 hours after 70 % hepatectomy (34,42,72).

Three peaks of hepatocyte DNA synthesis was reported by Wu et al. (82), initially in zone 1 and later in the mid-zonal “zone 2” of Rappaport’s classical lobule description. Approximately 15 % of hepatocytes do not enter into cell division meanwhile 11 % of them divide by three fold. The underlying mechanism of this phenomenon remains elusive.

10. Besides brain, the liver is the organ with major capacity of reacting to broaden internal perturbations and keeping its structure and function as well. This quality is also present in several biological systems just as: Physiologic homeostasis, tissue development, cell-cycle, ecological resilience, circadian rhythm, and even cancer development (133-135).

This phenomenon was coined as “robustness” and has concerned great interest in evolutionary biology in the last decade. LR shares all the robustness common features: Pleomorphism, redundancy and biofeedback mechanisms.

This makes that LR’s global understanding is extremely difficult and as consequence therapeutic interventions on LR is still lacking (2,4,10,44).

**PHASES OF LIVER REGENERATION: PRIMING, PROGRESSION AND TERMINATION**

It is a merit of Nelson Fausto (1,2), who has integrated the cellular and molecular mechanisms of LR in a three-phase model. LR can be divided into three phases: An initial phase of induction or priming phase which corresponds to hepatocytes transition from quiescent state, or G0 to G1 phase of the cell cycle, which takes place in the first 4 h post-HPx. A second phase or progression phase corresponds with the transition from G1 to the completion of mitosis; and a third phase or “apoptotic” phase of tissue remodeling and finally return to the initial G0 phase. The above model has been assessed in different species and has the virtue of corresponding with the cell-cycle phases, from which some basic aspects are reviewed (32,69,136-138).

Cell-cycle is divided into four phases: G1, S, G2 and M according with figure 5. DNA synthesis takes place in S phase (from synthesis). The chromosome segregation occurs in the M phase or mitosis, which is divided into four
stages: Prophase, metaphase, anaphase and telophase (the cellular division ends with the cytokinesis giving rise to two daughter cells, which can reinitiate the cell cycle or return to the previous G0 state). G1 and G2 phases also known as gap phases take place before DNA synthesis (S phase) and mitosis (M phase) respectively, adding extra time for cellular growing and surveillance of the transition to the following phases according with the extracellular and intracellular signals (69,137-139).

The cell-cycle has three control checkpoints: restriction or “R” check-point which defines the entry into the cycle in late G1 phase; and the two mitotic control check-points: The G2/M check-point which regulates mitosis entry and the metaphase-anaphase transition control check-point where the final mitosis events are initiated giving rise to the sister chromatides and mitosis cessation (69,70,136,140,142). The control “R” check-point regulates the rate of cell division and determines the cell cycle irreversibility, at whatever time cell progress beyond “R” point (69).

The majority of mitogens and growth factors- such as, platelet derived growth factor (PDGFG), EGF, HGF, and transforming β growth factors regulate the rate of cell division in the “R” check-point, when the cell is more sensitive to external factors (138-140).

INITIAL PRIMING PHASE
Sterile inflammatory response

According with the mentioned model, LR represents a physiological response to liver damage (63,143). The tissue injury can be expressed as “cellular stress” or as one of the knowing forms of cellular death: Necrosis, apoptosis or necro-apoptosis (58,144-147). Necrosis induces the recognition of DAMPs by the pattern recognition receptors (PRRs) which triggers the innate immune system. PRR’s receptors have been described in the cell membrane, endosomal membranes and in the mesenchymal and hepatocytes cytoplasm as well (44,54,55,61,62,148,149).

The regenerative response could restore the threatening situation or fail, depending of tissue injury severity. In the latter condition, liver is unable to restore the homeostasis, being associated with a high mortality and requiring in most of the cases a liver transplant as definitive solution (example: Fulminant hepatic failure, small-for-size syndrome, acute-on-chronic liver failure, or posthepatectomy liver failure) (150-152). In the above mentioned situations a progressive cholestasis, coagulopathy, encephalopathy, sepsis and multiple organ failure takes place. Some authors have related it with an innate immune “paralysis”; others have suggested that an excessive mitogenic stimulus could provoke a “hyperproliferation stress response” which leads to cellular apoptosis and failing liver (153-155).

In 1994 Polly Matzinger described the DANGER theory by which the natural immune system, besides recognizing pathogen germs and their wall components also recognizes the endogen ligands released by cellular damage and necrosis, giving rise to the denominated “sterile inflammation response” (54,55,59,156-158).

Necrotic cells leak the intracellular content –DAMPS– out to the extracellular compartment where bind to the patron receptors PRRs. Among those receptors belong the complement system and Toll-Like receptors (TLRs) which are expressed in the membranes and in the cytosol of macrophages, sinusoidal endothelial, dendritic cells, natural killer (NK) and hepatocytes (159-163) (Fig. 6). DAMPS are also recognized by a subsets of stress and damage receptors, known as NLRP which belong to the NOD-like receptors (NOD). Once they are triggered, give raise the “inflammosoma” complex, releasing pro-inflammatory and mitogenic cytokines such as: interleukin-1β (IL-1β) and interleukin 18 (IL-18). The NLRP receptor role has raised great interest in recent years regarding the mechanism of inflammation and survival (164-166).

Since the DANGER theory description by Matzinger, new tissue injury related DAPMs have been described just as: high mobility Group Box 1 (HMGB1), heat shock proteins (Hsps, Hsp60, Hsp70), uric acid, genomic DNA, ATP, adenosine heparin sulfate, oligosaccharides, degradation fragments of hyaluronic acid, mitochondrial N
formulated peptides, which have been denominated with the general term as “alarmines” (59,63,167-169).

It is indicative that recognizing proteins involved in the innate immune system are phylogenetic previous to the separation between vegetals and animals (thousand of million years ago) and to the acquisition of the adaptive immune system. On account of the semnal description of TLRs in Drosophila Melanogaster and in humans, as well as the DANGER theory, the paradigm of the innate immune system response and tissue regeneration has been reported (170-176).

The interaction between TLRs and their ligands triggers several signaling cascades which generate IL-6, TNF-β, IL-1β synthesis, and the activation of cytosolic transcription factors (NF-Kβ, STAT-3, AP-1) conveying the pro-proliferative stimulus to the nucleus (62). TLRs regulate the regeneration process in colon intestinal mucusa, lung, skin and post-hepatectomy regenerative response too by eliciting pro-survival signals and apoptosis inhibition too (63,170,177-179).

LR has also related with the endogenous ligands present in portal blood: Bile acids (BAs), xenobiotic, LPS and components of extracellular matrix-fibronectin, heparan sulfate, fibrinogen, hialuronic acid oligosaccharides- released by tissue injury (surgical manipulation) (180-185).

The nonparenchymal cells –specially macrophages–synthesize pro-inflammatory cytokines just as: IL-1β, TNF-α, IL-6, gamma-interferon (IFN-Y), protaglandins and activating platelet derived factor with antiapoptotic and proliferative functions (63,167,186-188). TNF-α and IL-6 cytokines initiate the “priming” phase of hepatocytes (transition G0-G1 of cell cycle). These cytokines signalize through the TNF receptors (TNF-R1, TNF-R2 and IL-6 (IL-R/gp130)). They are tyrosine kinase receptors similar to growth factor receptors, which lead to enzymatic cascades as the mitogen-activated protein (MAP) kinase cascades. Janus Kinase –JAK– is part of this group, which upon activation phosphorylates preformed transcription factor STAT-3 (JAK/STAT signaling). STAT-3 protein binds to the DNA activating the immediately-early response genes expression (IEGs): c-fos, c-jun, c-myc (known as oncogenes) which promote the cell-cycle entry (Fig. 6). More than 180 of these genes have been described, which synthesize the required protein to leave the quiescent G0 state (1,4,5,42,44,72,118,188-191).

The priming phase initiates in the first 30 minutes longing for the first 4 hours post-hepatectomy. In addition to STAT-3 factor, others involved transcription factors are Kappa-β nuclear factor (NF-Kβ) and the activating protein 1 (AP-1) required for the “novo” synthesis of G0-G1 and G1/S regulation proteins. The STAT-3, NF-Kβ and AP-1 participation were assessed in conditional KO animals, with genetic manipulation techniques (192-195).

Animals with mutations in TNF-α receptor (TNF-R1), showed an inhibition of NF-Kβ transcription and a severe deficit in liver regeneration, confirming that NF-Kβ transduction is crucial in the Kupffer cells response to cytokines. KO mouse for IL-6 receptors (gp 130) showed minor defects in cellular proliferation. In KO animals for IL-6 (IL-6 –/–) and its receptor (gp130), LPS administration following a partial hepatectomy significantly decreased the survival; confirming that IL-6 has a protector role in regeneration. Hepato-specific deletion of STAT-3 and AP-1, decrease the cyclin expression; which is mandatory for the G0-G1 transition in the cell cycle. On the other hand, when NF-Kβ factor was up-raised by blocking its cytoplasmatic inhibitor IkβKβ, a more intense inflammatory and proliferative response was assessed (72,196).

In addition to the mentioned inflammatory mediators-TNF-α, IL-6, C3a, C5b, LPS, growth factors as HGF, PDGF and EGF, also stimulate G0-G1 transition. The HGF signalizes through the C-met receptor, and activates the immediately early response genes. Mouse with a conditional mutation in Met receptor, showed a defect in LR (6,197,198).

As it will be described in the proliferation phase, HGF regulates gene expression of other cell cycle phases and exerts “pro-survival” effects by inhibiting apoptosis (4).

Complement system and liver regeneration

In mouse models of liver injury CI4C, partial hepatectomy and in patients submitted to a hepatectomy, a rising in the activated fractions of C3a and C5a were described in the first 24 hours post-procedure (131,132,149). Deficient mouse (KO) for C3 fraction (C3 –/–) and C5 fraction (C5 –/–), showed an impaired regeneration after 70 % hepatectomy. In these animals a decrease in the TNF-α, and IL-6 mRNA levels were assessed, in addition to a reduction in NF-Kβ and STAT-3 transcription factors (172-174). C5a receptor (C5aR) antagonist administration, provoked similar effects increasing the mortality; verifying the role of the complement in the initiation of the priming phase (199).

Both C3 –/– and C5 –/– KO animals developed more severe liver lesions than control animals following CI4C infusion or following a PHx. The regeneration impairment and hepatocellular injury were restored when C3a and C5a were administered. The same phenomenon was observed in double knockout animals (C3/C5 –/–) as well as regeneration recovering after C3 and C5 fractions administration.

Lambris’s group (170) has reported that C3a fraction triggers the IL-4 synthesis by NK/T cells in the first 24 hours post-PHx and elicits the complement protein synthesis by macrophages during the priming phase. Besides the elimination of pathogens and tumor cells, complement system represents the more speed humoral component for recognizing tissue injury through DAMPs exposure and triggering the reparative response (170,199).
Platelets and liver regeneration

In addition to the well known haemostatic functions, platelets express Toll-like-receptors, 2, 4 and 9, and participate in the innate immune response. These receptors recognize the previous mentioned PAMPs and DAMPs, by which some authors refer them as the “circulating guardians” of tissue injury similar to the hemocyte vestige in the invertebrates (200,201).

Platelets contain fibrinogen, Von Willebrand factor, adhesion proteins, angiogenic factors, hepatoprotective factors (VEGF, PDGF, HGF, IGF, EGF-1 and TGF-β) and 95 % of the circulating serotonin! Although they are non-nucleated cells, they have ARN and retain the synthesis capacity of more than 300 different proteins, among highlights the TGF-β (202,203).

In 1996 Tomikawa et al. (204) described that the thrombocytosis induced by a splenectomy significantly enhanced liver regeneration in rodents. Some findings were confirmed by other authors as well the opposite effects secondary to thrombocytopenia. The same group described an improving survival in a model of sublethal hepatectomy (> 90 % resected parenchyma) model by inducing thrombocytosis. Other groups have related platelets with a protective effect on the post-hepatectomy liver dysfunction and operative mortality with a threshold platelet count < 100,000/μL. The mentioned authors related these outcomes with the above cited growth factors and the insulin-growth factor-1 (IGF-1) (205,206).

Lesurtel published in 2006 the enhancing effect of serotonin in hepatic regeneration “in vivo” although its mitogen effects were already known in fibroblasts and hepatocytes “in vitro” (207). The confirmation of these findings were reported by the same group using KO animals for serotonin synthesis (TPM1 –/–) as well as improving survival following the administration of synthetic serotonin agonist (5HT2B) in a small-for-size murine model. The authors reported an increase of the endothelial fenestrations of grafts in the treated animals (208).

The same authors described the sinusoidal “pseudocapillarization” reversion in old animals as an increase in the survival rate following the agonist administration after PHx; suggesting that opening of the sinusoidal-endothelial fenestration would facilitate the direct contact of platelets, cytokines and growth factors with the hepatocytes (208). Others studies have related the serotonin proliferative effect with VEGF synthesis. Although Matondo et al. (209) did not observe any effect in a murine model of deficient membrane carrier, KO (TOH1 –/–) after 70 % PHx.

Cytokines, growth factors and hormones

In 1967, Moolten and Bucher reported that the plasma from previously hepatectomized rats induced mitogenic effects on hepatocytes “in vitro” as well as “in vivo”. Since then, several cytokines, growth factors, hormones and metabolites have been associated with the hepatocytes and mesenchymal cell cycle (4,210-213).

Family IL-6 cytokines

The IL-6 is an interleukin type I, associated with the innate immune response. Due to its cytoprotective, proliferative and anti-apoptototic effects is the most related with LR. In addition to IL-6 another interleukins sharing the Tyr-Kinase-coupled gp80 and gp130 receptors and signal transducing pathway have been described and referred as IL-6 family cytokines: IL-6, interleukin-11, leukemia-inhibitory factor (LIF), oncostatin (OSM), ciliary neurotrophic factor (CNTF), cardioprophin-1 (CT-1) and IL-27 (186-188,214-217).

IL-6 is rapidly secreted by macrophages during the initial injury tissue response. IL-6 exerts local effects on hepatocytes as well systemic-fever, somnolence, ACTH and vasopressin secretion –inherent to the stress inflammatory response. IL-6 induces the acute-phase protein synthesis by the liver (reactive C-protein, A-amiloid) and exerts similar inflammatory effects to TNF-α and IL-1 (218-221).

IL-6 binds to gp130 membrane receptors, provoking the dimerization of two gp130 subunits (222). Each cytokine receptors associate with cytoplasmic Tyr-Kinases of the Janus Kinase family, termed Janus type (JAK1, JAK2, JAK3 and Tyk2-Tyrosine Kinase 2). Thereupon gp130 dimerization and its binding to the receptor (IL-Rα/β) promote JAK kinase activation which activates the STAT-3 (Signal Transducers and Activators of Transcription) transcription. The STAT proteins (7 in the human being) localized in the cytoplasm, migrate inside the nucleus, bind to the DNA promoter and activate the transcription genes related with cell proliferation, survival/apoptosis (223,224) (Fig. 6).

The activated STAT-3 induces the transcription of immediate-early genes c-jun, c-myc, c-fos and the early growth (EGR-1). Moreover the IL-6/KAK-STAT-3 signaling pathway provides the antia apoptotic protein Bcl-2, Bcl-x synthesis. The KAK-STAT pathway is one of the more straightforward strategy of signaling transduction from the cell membrane to the genome. Hepatospesific deletion of STAT-3 decreases the cyclin D1 and E1, both required for the cell cycle entry (72,191,192).

Besides STAT-3, there are two other transcription pathways mediated by Tyr-kinases receptors, the nuclear factor kβ (NF-Kβ) and the activating protein 1 (AP1). The NF-Kβ factor usually remains inactive in the cytoplasm by the inhibitory protein IkbKβ. The cytokine binding to the transmembrane receptor triggers IkbKβ degradation, releasing the NF-Kβ factor which translocates to the nucleus and stimulates the acute phase protein syn-
thesis and the mentioned pro-proliferative and antiapoptotic genes. A rapid increase in TNF-α and IL-6 levels takes place after a 70 % PHx, or secondary to liver injury (ischemic, Cl4C, LPS administration). KO mice for IL-6 (IL-6 –/–) suffered hepatic failure following PHx, which was reversed upon previous administration of IL-6 (225). STAT-3 activation was almost absent in IL-6 –/– animals.

The STAT-3 protective effect on tissue injury was confirmed in KO conditional models, following adenovirus exposure (otherwise STAT-3 model is lethal in the embryo period) (198). The IL-6 cytoprotective effect was reported in diverse murine models of hepatotoxicity as ischemic-reperfusion injury or severe cholestatic. The IL-6 antiapoptotic effect was recently proved in extreme hepatectomies (87 % parenchyma resected) as well as in the rapid regeneration of “small-for-size” syndrome model (226-229). The AP-1 transcription factor promotes the immediately-early genes expression during the first five posthepatectomy hours; particularly the Jun oncoprotein which facilitates the G0-G1 transition of the cell cycle (72).

One of the IL-6 family cytokines, which has concerned particular interest is the cariotrophin-1 (CT-1). Prieto’s group has described its protector effect in apoptosis as well as a regenerative inducer in ischemic-reperfusion injury, in an extreme PHx (92 % resection) and in fulminant hepatic failure experimental model (230-232).

Metabolic and xenobiotic signaling receptors

In addition to the extraordinary capability of the hepatocytes for entering into the cell cycle, meanwhile the specific metabolic functions ought to be performed by the remnant liver along the process (2,3,4,238-240). The related metabolic gene expressions are initially supplanted by the genes involved in the cytoskeleton formation, the mitotic spindle assembly and mitosis. Transcriptional studies have shown a temporary silencing of the metabolic genes (first 40 post-hepatectomy hours) and a posterior recovering towards the end of mitosis (42,233).

It has been previously described that the bile acids, drugs detoxification and carbohydrates metabolism take part in the early priming phase. The tightly regulation of bile acids pool by the liver through the enterohepatic circulation is very well known (234). This pool is maintained in the human between 2 and 4 g, circulating about 12 times per day; in the mouse is kept in 4 mg (235). The bile acid increasing damages the cell membranes and mitochondrial too, promoting cell apoptosis and necrosis (236,237).

The bile acids homeostasis, is regulated by nuclear receptors, through “Farnesoid X-receptor” (FXR). Free and conjugated bile acids binding with FXR, stimulates the transcription factors involved in early regeneration process (G1-S transition) as FoxM1b and proliferative genes (Cdc 25) (166,180).

At the time of a 70 % PHx an abrupt increase in the portal flow to the remnant liver (1/3 of original mass) undergoes increasing by three fold the nutrients, bile acids and all contents present in the splachnic circulation (2,3,4,180,238-240).

Deficient mice in FXR suffer a delay in LR and higher mortality rate following PHx (180). These animals are unable to activate the early response genes c-myc, c-fos and c-jun. On the other hand the bile acids administration stimulated LR in previously hepatectomized mice and was delayed after cholesteramine administration.

The bile acids stimulate the pro-inflammatory cytokines as TNF-α and IL-1 by the kupffer cells with pro-survival, anti-apoptotic and proliferative effects (240). Bile acids and xenobiotics could behave as true DANGER molecules triggering the priming phase in addition to their mitogen effect, particularly the hydrophobic.

There is evidence that the administration of FXR receptor agonist enhance LR in old animals. These findings as their cytoprotective properties have raised its likely clinical application (241,242).

The relationship between the metabolic pathways and regeneration is complex given that the metabolic variations could be a consequence of the silencing of metabolic genes process without implying a direct mitogen effect. By the way, some authors have reported that persistent hypoglycemia in heptatectomized mice stimulates regeneration, meanwhile the glucose addition inhibits LR (42,243). Those findings seem to be contradictory with Fissette (244,245), who reported that glucose and insulin infusion previous to a major resection improved the liver dysfunction and regeneration.

Liver ability to response to endogen stimuli –bile acids– and xenobiotics is an exclusively feature of the liver which could explain its almost unlimited capability for a “robust” response to a wide range of stimuli as liver resection, ischemia or bile acid overload (4,42,240).

PROGRESSION PHASE

Mitogenic signal transduction

Although there is not a clear limit between the LR phases; the progression phase takes place since the end of the G1 phase to the cellular division or cytokinesis (2,4,5,9). Besides the chromatine, other cytoplasm organelles –mitochondria, Golgi apparatus, lysosome, endoplasmic reticulum– ought to duplicate during this period in order to maintain the ratio between the cytoplasm and nucleus size in advance of accomplishing the metabolic functions of the liver (69,73,109,110).

The progression phase is regulated by the previous mentioned growth factors –HGF, EGF, VEGF, and TGF-α– which as well their mitogen effects, exert trophic and pro-survival functions.
The primary growth factors role is to activate the Cdk- cyclin complex which evokes a wide variety of cell cycle changes: Mitotic spindle duplication and DNA replication in the S phase. The majority of the growth factors convey the mitogen signal to the nucleus through the membrane and cytoplasmic tyrosine-kinase receptors (RTK) promoting the downstream kinase cascade. The MAP-kinase cascade induces the immediately-early genes expression (c-fos, c-jun, c-myc), which is the first transcriptional event (two hours post-PHx). Among the factors codified by the pro-mitotic genes highlight FosM1b and Myc factors (1-6,42,43,246).

The FosM1b factor increases in the first post-PHx hour and evokes the activation of a large family of transcription factors termed AP-1, which stimulates the late response genes expression (two-days post-PHx). These genes regulate G1 cyclins (D1, D2, D3) required for the entry in the cell cycle (42). KO mice for c-jun, show 50 % mortality after PHx. The regeneration impairment is associated with an increase in cell necrosis and hepatocyte lipid accumulation. An increasing in the inhibitory G1-S transition protein has been described.

The Fos M1b factor promotes the late response genes expression (2nd post-PHx day) and the G2-M transition. The role of this factor in the entry into mitosis and in the chromosome segregation was confirmed in hepato-specific FosM1b KO mice (247). It has been reported that hepatocyte over-expression of FosM1b restored the injured liver, more efficiently than control hepatocytes (wild type), suggesting eventual therapeutic applications in old patients with liver diseases (248).

The Myc factor remains elevated throughout the entire cycle and promotes the “R” control point transition as well as the gene expression involved in the cellular size and metabolism (108). The Cyclin D levels remains elevated throughout the cycle meanwhile the mitogen is present with independence of the cell cycle phase (108). Growth factors and hormones such as insulin signalize through the Ras kinase cascade –MAP–, which phosphorylates and inhibits the glycogen synthase kinase (GSK3\(^{\beta}\)) activity, allowing glycogen synthesis and also the activation of the preformed factors –AP-1, Myc– which stimulate cellular proliferation.

Hassanain et al. (244,245) has recently described the benefit effect of insulin and dextrose infusion in patients submitted to a major hepatectomy. These authors have assessed glycogen liver increasing as well as an improvement in the early postoperative liver function. Besides the glycogen protector effect as energy source for cellular division, GSK3\(^{\beta}\) inhibition enhances the gene expression required for the regenerative response. The trophic effect of pancreatic hormones on liver parenchyma and liver regeneration was described by Starzl et al. in 1967 (249-252).

**Hepatocyte growth factor (HGF)**

The HGF was discovered in 1984 as a plasma protein with mitogenic effect in hepatocytes “in vitro”, from hepatectomized rats and initially denominated “hepatopoietin” (235). Given its mitogen effects “in vivo” and “in vitro” is the growth factor, which has raised more attention. In addition to the mitogenic effect have been reported diverse effects as: Mitogenic (enhancement of cell motility), trophic, antiapoptotic, angiogenic and morphogenic effect on liver, brain, placenta, lungs, intestines, myocardium and reproductive system (211,253,254).

The HGF is synthesized by mesenchymal cells as a precursor (pro-HGF) molecule and stored in the extracellular matrix (ECM). HGF is a glycoprotein very similar to the blood coagulation cascade factors and fibrinolysis as well (plasminogen). Pro-HGF activation involves urokinase-type plasminogen activator (U-PA) (2,4,69,255-261).

Following a hepatic injury the plasma levels of HGF increase very rapidly by 10 to 20-fold. The HGF plasma raising is derived from the stored HGF and the releasing by the macrophage synthesis upon IL-6 and TNF-\(\alpha\) stimulation. In addition HGF transcription increases in the mesenchymal cells of lung, kidney and spleen confirming its endocrine function (2,4,259,260,262-265). HGF binds to the tyrosine kinase receptor –C-met– which its phosphorylation is assessed during the first 1 to 15 minutes post-PHx, with the largest increase at 60 minutes. Following hepatic resection prolonged increasing in HGF plasma levels were observed, and remained elevated up to two weeks after liver resection. The higher HGF increasing has been reported in fulminant hepatic failure; this finding has questioned its therapeutic role in this condition. This paradoxical phenomenon -inability to stimulate cell proliferation with very high levels-, has been attributed to the C-met receptor inhibition by competitive effect by others ligands such as: IL-6, TGF-\(\beta\)1 or to the mentioned “hyperproliferative stress response” by which an excessive mitogen stimulus provokes an apoptotic response by p53 activation (61,266-270).

The HGF systemic and portal infusion in rodents increases DNA synthesis in the hepatocytes of zone 1. The infusion of human HGF into the portal vein in rodents resulted in hepatocyte proliferation and increase in relative liver mass (2,4).

The human HGF gene transfection by the hydrodynamic injection of plasmid DNA induces hepatomegaly in mouse via b-catenine activation, and the infusion of large amount of HGF increases the liver weight/body weight ratio associated with the mitogenic stimuli. The HGF withdrawal promoted apoptosis and the restoration of the basal DNA level (2,4,255,271).

The collagenase pretreatment in rats resulted in a higher response to HGF. Furthermore, isolated hepatocytes obtained by collagenase tissue digestion showed cell cycle initiating markers. This “pre-proliferative” pattern is
according with the competence acquisition phenomenon described by Pardee in 1989; by which the competent hepatocytes response steadfast to a posterior regenerative stimulus (1,4,73).

It has been proposed that the releasing of remodeling metalloproteinases from the extracellular matrix would contribute to the initial regeneration phase, sensitizing hepatocytes to the HGF stored in the liver. Likely the extracellular matrix remodeling by the urokinase and metalloproteinases, would generate a sterile inflammatory response by releasing DAMPs from tissue injury and consequently the binding to Toll-like receptors and activation of the transcription factors –NF-KB, AP-1 and STAT-3– involved in cell cycle entry (54,55,59).

The C-met membrane Tyrosine-Kinase receptor is present in epithelial and in mesenchymal cells. The C-met receptor binding with its ligand leads to down-stream kinase cascade activation by mitogens (MAP kinase); which stimulates the cytosolic transcription factors (AP-1) related with proliferation and cell survival.

In addition HGF also activates the Janus Kinase (JAK) pathway which activates the transcription factors such as STAT-3, nuclear factor Kb (NF-Kb) and β-catenin. The HGF binding with C-met receptor phosphorylates β-catenin giving rise to its nuclear translocation and up-regulation of target genes such as cyclin D required for the G0-G1 transition of cell cycle (2,4,188,264,267,268).

The role of HGF and its receptor in the regenerative response was also investigated using transgenic models. Mice lacking HGF and C-met receptor (null mice) die during gestation and express a reduced liver size and expression of the competence acquisition gene HB-EGF (283-285). In most of the tissues TGF-β inhibits proliferation at the G1 cell cycle phase (279-281).

Epidermal growth factor (EGF)

The EGF represents a family factors, from which high-light: Epidermal growth factor (EGF), amphiregulin (AR), heparin-binding EGF (HB-EGF) and transforming growth factor-α (TGF-α). These factors are over-expressed during a liver injury. They signalize through the tyrosine kinase receptors (RTK); EFR/ErbB1, HER2/ErbB2, HER3/ErbB3 and HER/ErbB4, that are collectively called EbbB1-4, which upon ligand binding promote downstream the mitogen activated cascade (MAPK kinase) (4,73,272).

Mouse lacking EGFR die between midgestation and postnatal day 20 with severe defects in the placenta, brain, skin and lung (273). In animals in which EGF was blocked and a partial hepatectomy was carried out; 1/3 died and the survivors showed a delay in hepatocyte division (274). However the liver regeneration was accomplished, suggesting that EGF signaling is important but not crucial for LR (61,274).

The rest of the EGF family are also synthesized as pro-factors that remain attached to the hepatocyte membrane and released by proteolysis. After PHx, the plasma levels of EGF rise, resulting in an increased EGF/EGFR ratio, confirming that EGFR plays a mitogenic role in the initial phase of LR (4).

The HB-EGF role was investigated in KO models and in transgenic animals as well. A delay in hepatocyte proliferation was observed in the first case and by contrast liver regeneration was stimulated in the second one. Animals lacking amphiregulin showed an impaired regenerative response, following a PHx (275,276).

The TGF-α is produced by the own hepatocytes –autocrine effect– and furthermore exerts paracrine effects on the endothelial and biliary cells. After HPx the TGF-α levels increases in the first 24-48 hours. The TGF-α addition to hepatocytes “in culture” and “in vivo” induces DNA synthesis and stimulates the transition through the control “R” point of cell cycle (279-281).

TERMINATION PHASE AND LIVER SIZE REGULATION

Liver regeneration ends when the initial liver mass is restored and enabling it to carry out the liver functions (liver index ≥ 2.5 %). The strict liver index regulation is one of the most intriguing feature of LR, because underlies a strict control at the end of the cell cycle and in the “neoformed” tissue remodeling process too (2,4,5,42,61,72,73).

The regeneration termination phase has been related with anti-inflammatory and pro-apoptotic cytokines as well as “hepatostat” factors such as IL-10, signaling cytokine suppressor proteins (SOCS-3), plasminogen activator inhibitor PAI-I and particularly the TGF-β (3,6,61,282).

Elegant studies using microarrays techniques in mouse and also in pigs have provided that the initial pro-mitotic gen over-expression is later supplanted by metabolic genes activation and afterwards by the cessation of proliferation and the returning to the previous quiescent state (34,41,61,72,73).

The transforming growth factor β family (TGF-β 1-3) comprises a number of cytokines related with cell differentiation and healing. They signalize through two receptors (type I and type II) which activate transcription regulators known as SMAD proteins (Small Mothers Against Decapentaplegic) which are translocated to the nucleus. In most of the tissues TGF-b inhibits proliferation at the G1 cell cycle phase (283-285).

Following a PHx an early increase in TGF-β ARNm has been described though a TGF-β transitory “resis-
mitogenic stimuli, reducing the rate of p53 ubiquitination. p16INK4a: Cdk E2F: Protein, whose concentration increases in response to excessive cycle arrest. modified from Morgan DO. RTK: Tyrosine-kinase receptor. activin receptor antagonist– following PHx, induced an complementary functions. The injection of follistatin –an activin receptor antagonist– following PHx, induced an increasing in the liver weight and in the hepatocyte proliferation, supporting that activin A inhibits the proliferation and takes part in regulating liver regeneration termination (5,61,73,292).

In hepatocyte culture and in “bioartificial” models, the extracellular matrix plays a significant role in hepatocyte differentiation and proliferation (60,61,73). In spite of representing only 0.5 % of liver weight, the ECM plays an active role in the tissue injury response and regeneration. Besides storing growth factors –PDGF, TGF-β, VEGF, HGF– the ECM contains cytokines, metalloproteinases (collagenase, gelatinase), and macromolecules as fibronectine, laminine and collagen (type I, II, and VI) very reactive whenever the endothelial-sinusoidal barrier is damaged (184,185,293-298). After liver damage –surgical trauma, ischemia, toxic– the type urokinase plaminogen activator (µ-PA) and metalloproteinase are released, stimulating the growth factors liberation as well as the macromolecules degradation (fibrinogen, fibronec tin, laminin) and eventually the DAMPs expression (DANGER theory) and promoting the sterile inflammation response and the hepatocyte priming phase (60,156,157,185).

The initial post-PHx proliferation associated with ECM degradation has been reported; giving rise to assembling nests of hepatocytes with deficient access to endocrine and paracrine factors, which in basal conditions inhibit cell division. The hypertrophic compiled hepatocytes require a posterior remodeling process up to get the initial hepatic index (72).

Michalopoulos group has corroborated the inhibitory effect of an integrin linked kinase (ILK) upon hepatocyte proliferation “in vitro” (ILK). Hepatoespecific KO animals for this integrin acquire hepatomegaly and enhanced hepatocyte proliferation (42,299). The hepatoespecific ablation of ILK impairs the regeneration termination. When these animals are submitted to a PHx the deficient livers in ILK reach a 58 % higher weight than the initial at 14 days post-HPx. The HGF and its C-met receptor expression increasing and the cell cycle inhibitor p27 diminution were observed (299-302).

Given the great interest in developing “bio-artificial livers” through the re-populating of previous decellularized matrix scaffold with hepatocytes and pluripotential cells, its development exploitation has been stimulated keeping in mind the increasing demand of other solid organs than the liver for transplantation as kidney, lung and heart (293-298,303,304).

**p53 and regeneration control**

Multicellular organisms contain cell cycle control mechanisms which react to stress situations –lack of nutrients, hypoxia, DNA damage– arresting the cell cycle or inducing cellular apoptosis (305,306).
The most prevalent system is the gen regulatory protein p53, also known as the “genome gatekeeper” by which cell responses to DNA damage or another stress conditions (307-311). Even though p53 is known as a tumor suppressor factor (more than 25,000 mutations have been reported in human tumors), p53 has recently raised great interest as regulator of LR ending (312).

Kurinna et al. has described that the gen p53 is necessary for the high grade ploidy and aneuploidy reverting, reported in basal conditions likewise during LR. Mouse with p53 lacking (p53 /−/) were associated with a higher ploidy grade (8n and 16 n) as a result of failures in the cell cycle final phase or cytokinesis. The same authors described for the first time that p53 regulates the expression of genes involved in the three cell division phases: Initiation-progression, division and restoration to the quiescent G0 state after PHx (117).

It is well known that p53 activation arrests cell cycle inducing apoptosis at the time of cellular stress conditions as severe hypoxia, acidosis or an excessive mitogenic stimulus. The latter is termed as “hyperproliferation stress response”. Culture cells after several divisions undergo a stable cell-cycle arrest induced by p53 rising. Cellular replicative senescence has been related with hyperproliferation stress –overactive Myc and Ras– or with non-physiological conditions given “in vitro”: Deficient extracellular matrix components and inadequate levels of oxygen. Additionally cells lacking p53 proliferate endlessly and are therefore called “immortal” (313-317).

Although the hyperproliferative stress response has been related with protective mechanisms against cancer development, this physiologic response could be the underlying setting of clinical situations in which an excessive mitogenic stimuli takes place as “small-for-size syndrome”, fulminant hepatic failure, post-hepatectomy liver failure or acute-on-chronic failure. In these conditions a large mitogenic stimulus is provoked by the endothelial damage, necrosis, parenchymal hemorrhage and hypoperfusion as a result of arterial vasospasm (150,151,318-320).

Today is still unknown why the liver regenerates in some cases and fails in others, developing an irreversible hepatic failure; not yet there is a clear limit regarding the minimal size remnant in liver resection or in liver-donor procedure (10,151,321-324). In spite of the myriad of papers regarding liver regeneration, its regulatory mechanism still remains as an enigma (2,4-6,9).

CONCLUSIONS AND FUTURE PERSPECTIVES

Liver regeneration still endures as one of the most amazing an enigmatic paradox of adaptation and response to keep the homeostasis. In the last decades has extensively investigated given its therapeutic consequences. LR is an extremely complex process, strictly regulated, which reproduces the tissue injury restoration, with an initial or priming phase -G0-G1 progress of hepatocyte cell cycle- and a posterior phase of proliferation-phases S and M- that ends with the hepatic mass restoration. This phenomenon has been related with hormones (insulin, glucagon), cytokines (TNF-α, IL-1β, IL-10), growth factors (HGF, EGF, VEGF) and in the last decade with the mesenchymal hematopoietic stem cells (CD 133+, CD117+) and in addition with oval cells. The current description of developing structures with “hepatoïd” phenotype derived from induced pluripotential stem cells (iPS) in rodents, has raised great concern as a future alternative to liver transplantation; although because the liver complexity should be seen with caution (325,326).

LR rigorously accomplishes the characteristics of the most regulated biological systems (robustness) –pleiomorism, redundancy and feedback mechanisms– such as the cell cycle, natural immune response or circadian rhythm, which illustrates the difficulty in its guidance for therapeutic goals.

Because the liver functions complexity, the intents of definitive or temporarily supplying methods have been elusive, with exception of liver transplantation. Perhaps cell therapy development and acknowledge of liver regeneration molecular and physiological basis could get near the expected dream of using the own liver regenerative capacity in the treatment of liver disease, which at present only liver transplantation is a real option.

Santiago Ramon y Cajal aphorism, “It is fair to say that, in general, no problems have been exhausted; instead, men have been exhausted by the problems” is very opportune regarding liver regeneration unveiling efforts.

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