ALPPS

From Human to Mice Highlighting Accelerated and Novel Mechanisms of Liver Regeneration

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Objectives: To develop a reproducible animal model mimicking a novel 2-staged hepatectomy (ALPPS: **A**ssociating **L**iver **P**artition and **P**ortal Vein Ligation for **S**taged Hepatectomy) and explore the underlying mechanisms.

Background: ALPPS combines portal vein ligation (PVL) with liver transection (*step I*), followed by resection of the deportalized liver (*step II*) within 2 weeks after the first surgery. This approach induces accelerated hypertrophy of the liver remnant to enable resection of massive tumor load. To explore the underlying mechanisms, we designed the first animal model of ALPPS in mice.

Methods: The ALPPS group received 90% PVL combined with parenchyma transection. Controls underwent either transection or PVL alone. Regeneration was assessed by liver weight and proliferation-associated molecules. PVL-treated mice were subjected to splenic, renal, or pulmonary ablation instead of hepatic transection. Plasma from ALPPS-treated mice was injected into mice after PVL. Gene expression of auxiliary mitogens in mouse liver was compared to patients after ALPPS or PVL.

Results: The hypertrophy of the remnant liver after ALPPS doubled relative to PVL, whereas mice with transection alone disclosed minimal signs of regeneration. Markers of hepatocyte proliferation were 10-fold higher after ALPPS, when compared with controls. Injury to other organs or ALPPS-plasma injection combined with PVL induced liver hypertrophy similar to ALPPS. Early initiators of regeneration were significantly upregulated in human and mice.

Conclusions: ALPPS in mice induces an unprecedented degree of liver regeneration, comparable with humans. Circulating factors in combination with PVL seem to mediate enhanced liver regeneration, associated with ALPPS.

Keywords: ALPPS, PVL, Transection, novel mechanisms of regeneration, human to mice

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Large or multiple liver tumors cannot be removed in many patients because of insufficient size and function of the putative future liver remnant (FLR).[1,2] When extended liver resections are attempted, the postoperative course can be complicated by the development of liver failure, so-called "small for size syndrome" (SFSS),[1,3] which often leads to death of the patient. Surgical strategies combining 2 operations (staged hepatectomy), including right portal vein occlusion and wedge resections of all left-sided tumors in a first step, followed by extended right hepatectomy in a second step 1 month or more later, are currently considered as standard therapy by many.[2,4] Portal vein occlusion can be achieved either by portal vein ligation (PVL) or by portal vein embolization. A drawback of this approach is the need for long time intervals between the 2 steps, which increase the risk of disease progression and render the second operation difficult because of the development of diffuse adhesion.[5] Another shortcoming in some cases is the inadequate hypertrophy of the FLR and the risk of SFSS after the completion hepatectomy during the second step.[1] Both drawbacks lead to a 30% to 40% dropout with patients, who may not benefit from a curative resection.[6,7]

A novel 2-staged surgical procedure with the nickname "ALPPS" for **A**ssociating **L**iver **P**artition and **P**ortal vein ligation for **S**taged hepatectomy[8] was introduced in 2011 to overcome those most feared complications, that is, insufficient FLR and long interval between both steps.[8,9] In ALPPS, PVL is combined with liver transection during the first step, followed by resection of the deportalized liver (*second step*) a few days later. This technically challenging approach has triggered an unprecedented number of comments, publication of small case series, and propositions for modification because of consistently reported higher morbidity and mortality rates.[6,10–16] To better understand the underlying mechanisms of accelerated regeneration triggered by ALPPS, we developed the first model of ALPPS in mice and tested whether such mechanisms may also be active in humans.

METHODS

Animals

All animal experiments were performed in accordance with Swiss Federal Animal Regulations and approved by the Veterinary Office of Zurich. C57Bl/6 mice, aged 10 to 12 weeks, were obtained from Harlan (NL) and used for all experiments.

Development of the ALPPS Model in Mice (Steps I and II)

Anesthesia was induced by isoflurane inhalation (2%–4%). For PVL, portal branches of the caudate lobe, right lobe, and right middle lobe of the liver were individually ligated using 9-0 silk, as illustrated in Figure 1. To achieve a 90% portal occlusion of the whole liver, the small left lateral lobe (LLL) was removed after ligation of the pedicle with prolene 8/0. Both the portal vein and the artery of the left middle lobe, serving as the FLR and representing 9% to 11% of the total liver volume, were preserved. In the ALPPS group (step I), 90% PVL was combined with 80% liver parenchyma transection between the deportalized right middle lobe and the normally perfused left middle lobe, using a bipolar forceps (Fig. 1). A routine cholecystectomy was

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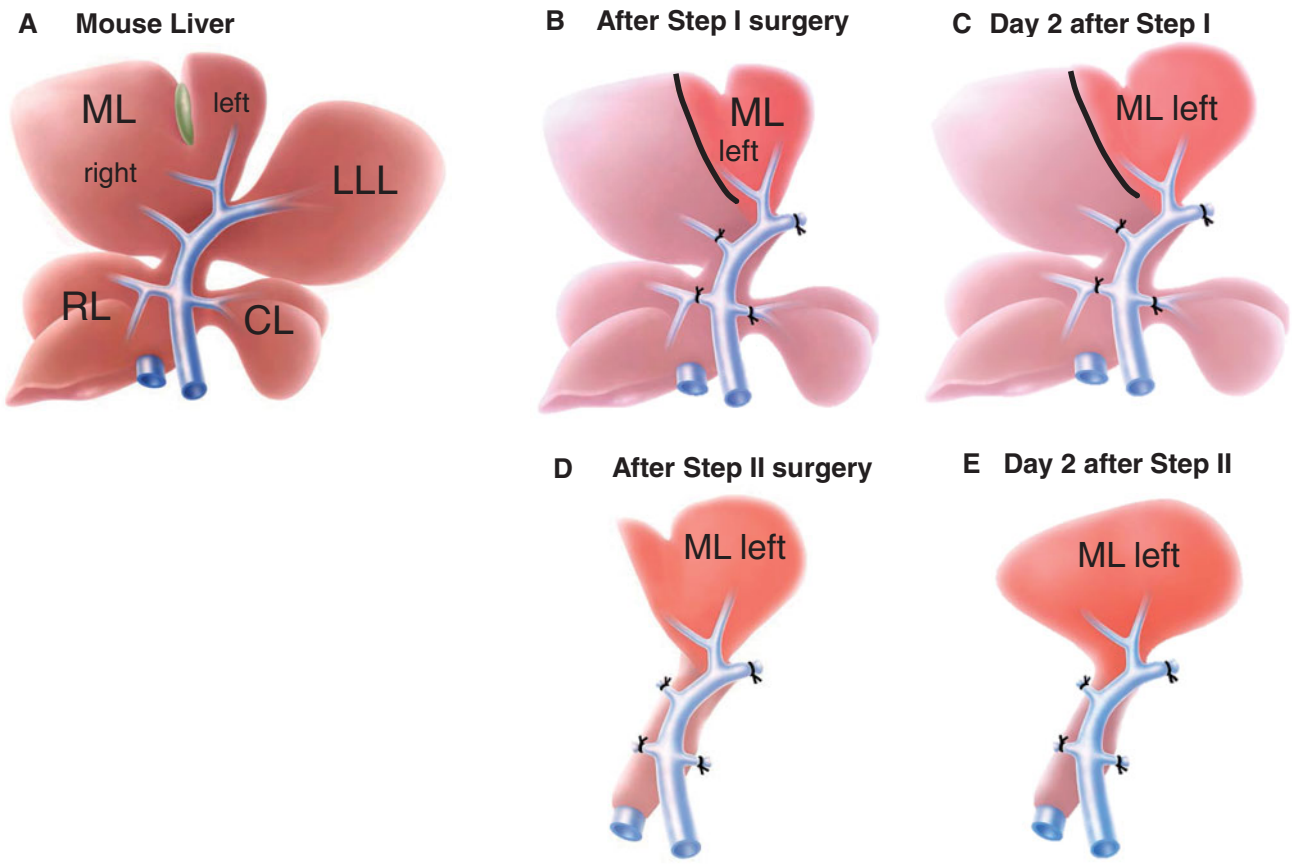

FIGURE 1. Surgical procedure of ALPPS (steps I and II) in mice. A, Baseline of mouse liver. B, Step I of ALPPS in mice; PVL performed at the caudate lobe, right lobe, and right middle lobe. Note the demarcation between normally perfused left middle lobe and portal-depleted right middle lobe, where transection is performed. In addition, the left lateral lobe is resected and standard cholecystectomy is performed. Both vessels of the left middle lobe remain untouched. C, Two days after step I, the left middle lobe shows significant increase in size, and step II including resection of all deportalized liver lobes is performed. D, Again, 2 days after step II, the remnant liver shows a significantly increased regeneration (E), whereas 7 days after step II, basically a new liver was developed, derived from initially 10% liver volume. CL indicates caudate lobe; ML, middle lobe; RL, right lobe, LLL, left lateral lobe.

performed during step 1 surgery. The following experimental groups for *step I* were included:

- ALPPS group: 90% PVL and 80% transection between right and left middle lobes.
- PVL group: 90% PVL alone.
- Transection group: 80% parenchymal transection between right and left middle lobes alone (as in ALPPS).
- LLL group: Resection of left lateral liver lobe. Animals in the LLL group served as negative controls.

Each animal was clinically observed, and liver tissue and plasma were obtained at days 1, 2, 4, and 7 after the first step (see Supplemental Digital Content Table 1, available at http://links.lww.com/SLA/A637). Ninety percent hepatectomy (caudate lobe, right lobe, right middle lobe, and LLL) led to the development of SFSS, and all animals died within 24 to 36 hours after surgery.

Step II

Animals underwent a relaparotomy 48 hours after step I to remove the deportalized liver lobes (caudate lobe, right lobe, and right middle lobe) (6-0 silk) (*ALPPS and PVL groups*). The same lobes were resected in both other control groups (*transection and LLL groups*) keeping only the left middle lobe (Fig. 1).

To explore the underlying mechanisms of regeneration of step I, some animals underwent 90% PVL with additional partial renal, splenic, or pulmonary bipolar ablation (mechanical burning) instead of liver parenchyma transection, using the same bipolar device (*PVL + kidney*; *PVL + spleen*). Pulmonary bipolar ablation was performed in the left lobe of the lung through a left-side thoracotomy, followed by 90% PVL and abdominal closure (*PVL + lung*). Furthermore, plasma was obtained from mice 1 hour after completion of ALPPS step I. This sample of plasma (200 μL) was injected in the infrahepatic vena cava in mice immediately after PVL but in absence of other manipulations. PVL-mice, injected with sham plasma sample, served as controls (*PVL + sham plasma*). To explore inflammatory and growth response after surgery, liver tissue and plasma samples were obtained 1 hour after step I surgery (*all groups; ALPPS step I, PVL, transection, LLL*).

Human Sample Collection and Analysis

To assess the inflammatory response in patients undergoing ALPPS, liver samples were prospectively collected at the end of the

surgery (*1 hour after ALPPS step I or PVL only*). Quantitative polymerase chain reaction (PCR) for interleukin-6 (IL-6), tumor necrosis factor α (TNF-α) (mRNA), was performed in these tissue samples. Plasma samples were collected at the same time to measure circulating IL-6 in the same patients (human ALPPS step I: n = 5; human PVL alone: n = 3). All samples were regularly included after proper ethical approval of the patients (clinical trials.gov, NCT 01775267).

Liver Weight to Body Weight Ratio

Liver regeneration was assessed by the left middle lobe weight to body weight ratio (FLR/BW) at days 1, 2, 4, and 7 after surgery (steps I and II). Animal weight was measured directly before harvesting. Ten animals were allocated per group and time point (see Supplemental Digital Content Table 1, available at http://links.lww.com/SLA/A637).

Histological Examination

Immunostainings were performed for Ki67 (Abcam), pH3 (Chemicon), F4/80 (BMA Biomedicals, Augst, Switzerland), and Cd31 (BD Pharmingen), using the Ventana Discovery automated staining system and the iView DAB kit (Dako North America Inc., Via Real Carpinteria, CA) and counterstained with hematoxylin. The number of Ki67-positive and pH3-positive hepatocytes was determined by manual counting in 20 random visual fields (200×).

Plasma Values

Plasma samples were obtained from the infrahepatic vena cava before organ harvesting. Plasma levels of alanine aminotransferase and bilirubin were measured using a serum multiple biochemical analyzer (Ektachem DTSCII; Johnson & Johnson Inc, Rochester, NY). Hmgb-1-Elisa was obtained from IBL (mouse: ST51011), Factor-V-Elisa from Cusabio (mouse: N2407272873), and IL-6-Elisa from R&D system (mouse: Dy406; human: Dy206).

Quantitative Real-Time PCR

Total RNA was extracted from liver tissue using Trizol reagent (Invitrogen, Basel, Switzerland). After generation of complementary DNA (ThermoScript reverse-transcription PCR system; Invitrogen), quantitative real-time PCR amplification and data analysis were performed on the ABI Prism 7000 Sequence Detector System (PE Applied Biosystems, Rotkreuz, Switzerland). *TaqMan* gene expression assays (PE Applied Biosystems) for IL-6 (mouse: Mm00446190_m1, human:Hs00985639_m1) and TNF-α (mouse: Mm0044 3258_m1;human: Hs99999043_m1) were used and normalized to 18S rRNA (control reagents; PE Applied Biosystems).

Statistical Analysis

Data are expressed as mean plus standard deviation. Differences between the groups were assessed by a Mann-Whitney *U* test. The level of statistical significance was set at $P < 0.05$. Statistical analyses were performed using Prism 4.0 (GraphPad).

RESULTS

New Model of ALPPS in Mice (Step I)

The development of the animal model followed, as closely as possible, the 2 steps of the ALPPS procedure (Fig. 1), as performed in human. Animals showed normal recovery and above 90% survival after surgery. The overall low mortality suggests that this new approach in mice does not induce liver failure, despite the application of 90% PVL. Although serum alanine aminotransferase levels increased after step I in ALPPS and PVL alone, we did not observe any sign of liver failure such as hyperbilirubinemia, low factor V, or increase in Hmgb-1 levels during the first week after step I (Fig. 2). Parenchyma of FLR did not disclose any abnormalities after step I (Fig. 2).

Based on these findings, we concluded that step I of ALPPS model is feasible, reproducible, and does not induce relevant injury in mouse liver.

Accelerated Liver Regeneration After Step I

We, serially, measured the weight gain of the FLR (left middle lobe) in relation to mouse body weight within 1 week after step I. The FLR/BW ratio indicated a significantly accelerated hypertrophy, reaching 100%, already by day 1 after step I. In contrast, doubling of FLR weight after PVL required at least 4 days (Fig. 3). The proliferative index, assessed by Ki-67, was significantly higher in ALPPS, when compared with other groups (Fig. 3). In addition, the ALPPS group displayed more hepatocyte nuclei positive for pH3, a marker of mitosis (Fig. 3). In contrast, the large nuclei, positive for pH3, were completely absent in mice after PVL or transection alone, as well as resection of the LLL (negative control) (Fig. 3). These results indicate that significantly more hepatocytes enter the cell cycle earlier after ALPPS than after PVL or any other groups. Of note, 1 week after step I, FLR after ALPPS remained 1.5-fold larger than after PVL (Fig. 3).

We conclude from this set of experiments that ALPPS induces an accelerated liver hypertrophy, supported by volume increase and hepatocyte proliferation.

ALPPS Procedure—Step II

In clinical practice, a major advantage of ALPPS remains the possibility for earlier completion of the step II operation, that is, removing the deportalized liver within 1 to 2 weeks after step I.[5] Animals allocated to ALPPS showed normal liver remnant to body weight ratio (LR/BW ratio) 7 days after the complete procedure (ie, 9 days after step I). The size of the remnant liver (left middle lobe) was comparable with native livers (Fig. 4). Of note, step II was feasible in all groups and led to 90% survival after ALPPS, PVL, or transection. Animals allocated to the LLL group (negative control), however, died from liver failure because of SFSS within 24 hours after step II (Fig. 4). During step I, these animals (LLL group) did not receive PVL or parenchyma transection, and consequently regeneration did not occur leaving behind a too small FLR.

We concluded from these experiments that liver growth was primed by transection, resulting in a superior liver hypertrophy than in any other groups.

Extrahepatic Injury Mimics the Effect of Transection in ALPPS

To explore whether transection triggers accelerated regeneration because of a discontinuity between the FLR and the rest of the liver or rather through the release of growth factors, we developed other models including partial tissue bipolar burning (ablation) of the kidney, spleen, or lung. In each of these groups, FLR increased in a similar range, as in the full ALPPS model including proliferative markers such as Ki-67 (Fig. 5).

ALPPS-Plasma Injection After PVL Triggers Comparable Regeneration, as Original ALPPS

Plasma, obtained from mice 1 hour after completion of ALPPS step I, was injected in PVL-treated mice alone. This maneuver induced a comparable degree of liver weight gain and hepatocyte proliferation, as in the ALPPS model (Fig. 5). In contrast, plasma obtained from animal after sham laparotomy failed to induce any additional regeneration in PVL-treated livers.

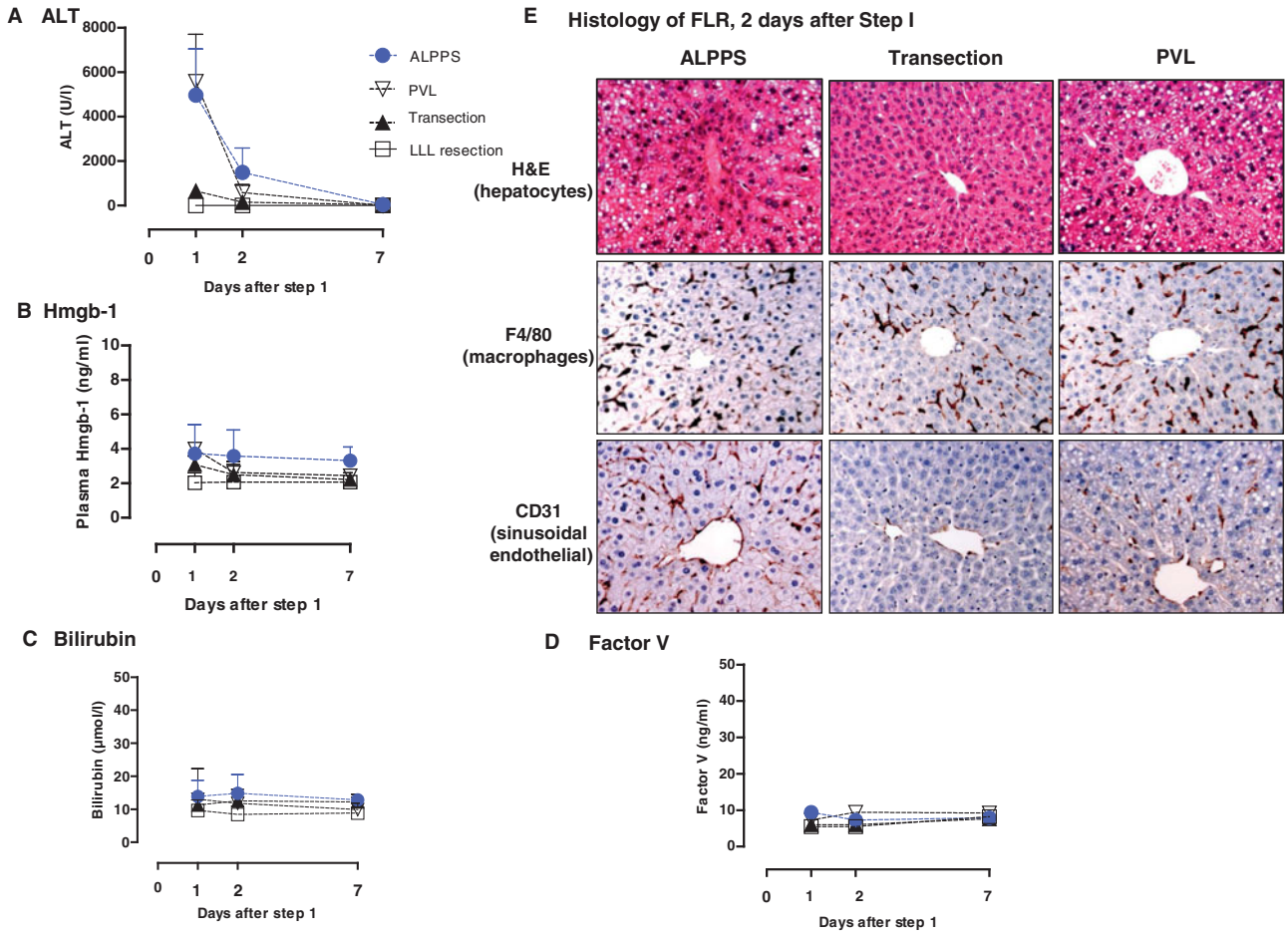

FIGURE 2. Markers for liver function and injury after step I of ALPPS, transection or PVL. Plasma alanine aminotransferase (A), Hmgb-1 (B), bilirubin (C), and Factor V (D) at 24 hours, 48 hours, and 168 hours after step I. Note the increased level of ALT in the early phase after ALPPS and PVL, while bilirubin remained low during the first week after step I. Partial necrotic cell death leads to an increase in ALT, derived from the portal-depleted liver lobes in ALPPS and PVL animals. These lobes contribute to a stable liver function, as detected by Factor V (D). The left middle lobe in ALPPS, with preserved vessels, showed no signs of necrosis (H&E staining, E) and exclusively liver steatosis was slightly different between ALPPS, PVL, and transection. Accordingly, macrophages were comparable stained using M1 marker F4/80 in all groups, and endothelial lining was not increased (CD31 staining) (E). ALT indicates alanine aminotransferase, Hmgb-1, High-Mobility-Group-Box-Protein-1.

On the basis of these experiments, we conclude that the rapid liver volume increase after ALPPS is triggered by systemic release of putative growth factors.

Soluble Initiators of Accelerated Liver Regeneration

After injection of ALPPS plasma in PVL-treated mice, we performed several analysis of plasma, obtained 1 hour after ALPPS surgery. At this early time point, animals after ALPPS contained elevated plasma levels of IL-6 relative to PVL alone (see Supplemental Digital Content Fig. 1, available at http://links.lww.com/SLA/A636). Mice, allocated to transection, showed a comparable increase of IL-6-plasma levels at this early time point. Accordingly, auxiliary promoters (ie, IL-6-mRNA and TNF-α-mRNA) were significantly upregulated in regenerating FLR 1 hour after step I of ALPPS. Transection alone triggered a comparable upregulation of the same genes at such early time points after surgery (see Supplemental Digital Content Fig. 1, available at http://links.lww.com/SLA/A636).

To explore whether similar mechanisms might be active in human, we used our prospectively intraoperatively collected blood samples from patients, who underwent either ALPPS or PVL. We compared gene expression of IL-6 and TNF-α in liver tissue and plasma levels of IL-6 1 hour after step I in patients who underwent either PVL or ALPPS. Genes related to the IL-6-TNF-α-STAT3-pathway disclosed a significantly higher expression after ALPPS step I than PVL alone. This finding was corroborated by a significant increase of IL-6 in plasma after ALPPS step I relative to PVL alone (see Supplemental Digital Content Fig. 1, available at http://links.lww.com/SLA/A636).

Findings in animals, consistent with clinical data, suggest a contribution of the IL-6-TNF-α-STAT3-pathway to the rapid liver hypertrophy after ALPPS step I.

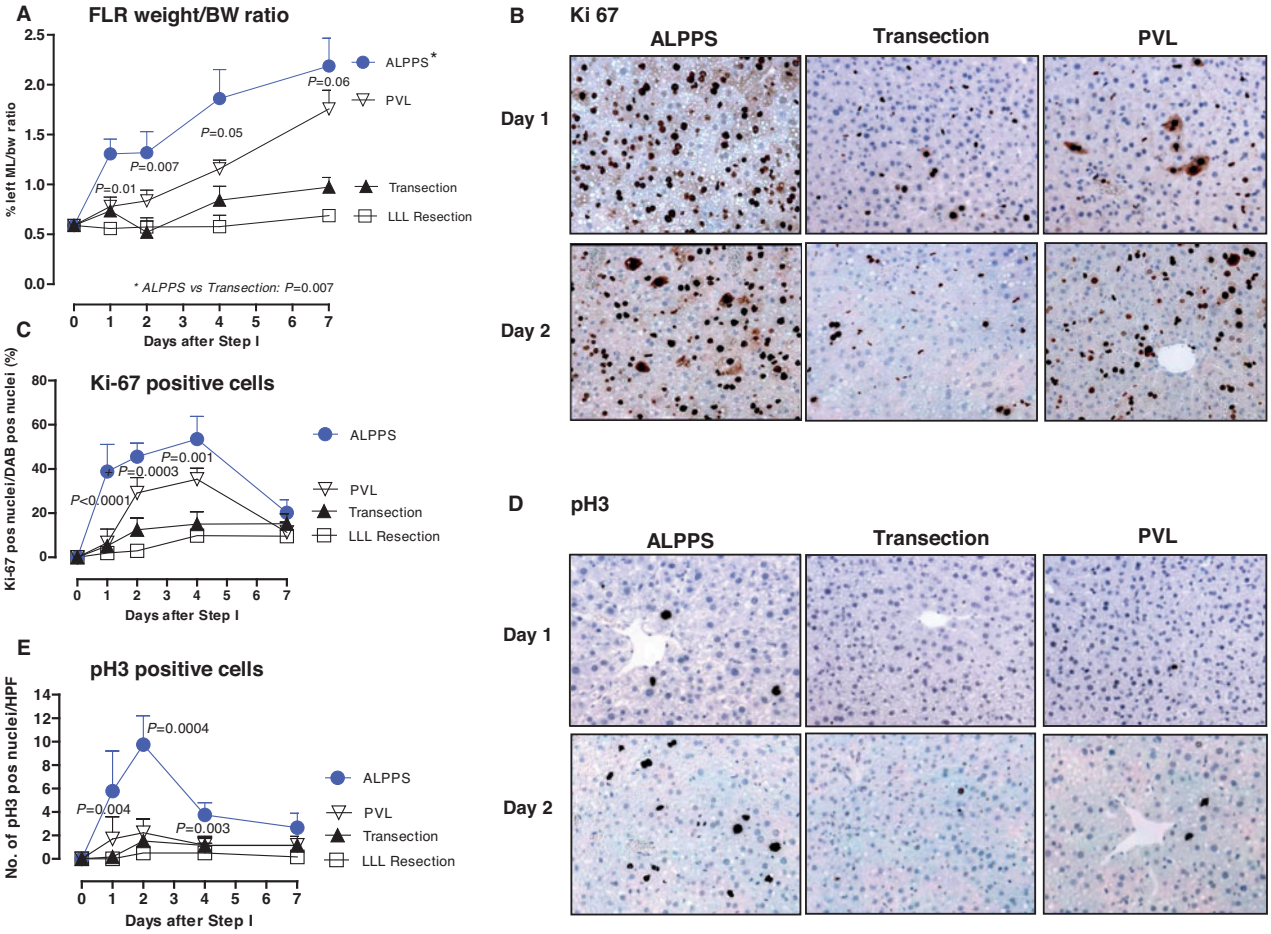

FIGURE 3. Volume gain and proliferation markers in future liver remnants during the first week comparing ALPPS, PVL, and transection. A, The FLR/BW indicates a significantly increased weight gain of left middle lobe already within the first 24 hours after step I of ALPPS as compared with PVL or transection alone (FLR/BW ratio: ALPPS vs PVL: day 1: $P = 0.01$, day 2: $P = 0.007$, day 4: $P = 0.05$). B, Liver sections 24 hours and 48 hours post–step I stained for Ki-67 and pH3 (D). Quantification of Ki-67 (C)- and pH3-positive cells (E) after step I in all different groups. Note the large amount of Ki-67 positive nuclei already 1 day after step I of ALPPS (Ki-67 positive cells: ALPPS vs PVL: day 1: $P < 0.0001$, day 2: $P = 0.0003$, day 4: $P = 0.001$) and prominent, bold nuclei in pH3 stains of ALPPS mice at 24 and 48 hours (D) (pH3 positive nuclei: ALPPS vs PVL: day 1: $P = 0.004$, day 2: $P = 0.0004$, day 4: $P = 0.003$), while PVL-treated livers present a markedly delayed increase of Ki-67 positive nuclei in accordance with decelerated volume increase as compared with ALPPS procedure. Error bars refer to ±SD. BW indicates body weight; ML, middle lobe; FLR, Future Liver Remnant.

DISCUSSION

The development of an ALPPS model in mice put forward 3 main findings. First, we confirmed the unprecedented and rapid regenerative capacity of a small FLR after step I of the ALPPS procedure. Second, we provide evidence suggesting that the accelerated regenerative ability of the FLR is due to circulatory growth factors, rather than a discontinuity of the microcirculation after liver transection. Third, preliminary data suggest that similar mechanisms are active in human.

ALPPS was developed to offer an increased chance of curative resection in patients with multiple or large liver tumors by inducing accelerated hypertrophy of the FLR and reducing the delay to completely clear the liver from tumors.[6,8–10] Although some authors confirmed the impressive and fast volume increase of the FLR after ALPPS,[8,9,11,12,15–18] others have repeatedly challenged the superiority of ALPPS in triggering liver regeneration, when compared with other approaches, such as portal vein embolization, particularly when segment IV (four) is included in the portal occlusion.[19,20] The first aim of the newly developed model of ALPPS was, therefore, to solve this issue. The model demonstrated that ALPPS is associated with accelerated growth of the FLR, along with all markers of hepatocyte proliferation, when compared with PVL alone. Interestingly, PVL itself has been previously shown to be equal or superior to portal vein embolization in inducing regeneration in rodent models.[21,22] Although the kinetic of regeneration is different in humans and rodents, the observation of accelerated regeneration is consistent with clinical data.[8,9,15,17]

There are ongoing debates concerning the underlying mechanisms behind the accelerated liver regeneration induced by ALPPS. Many have postulated that this effect relies on the discontinuation

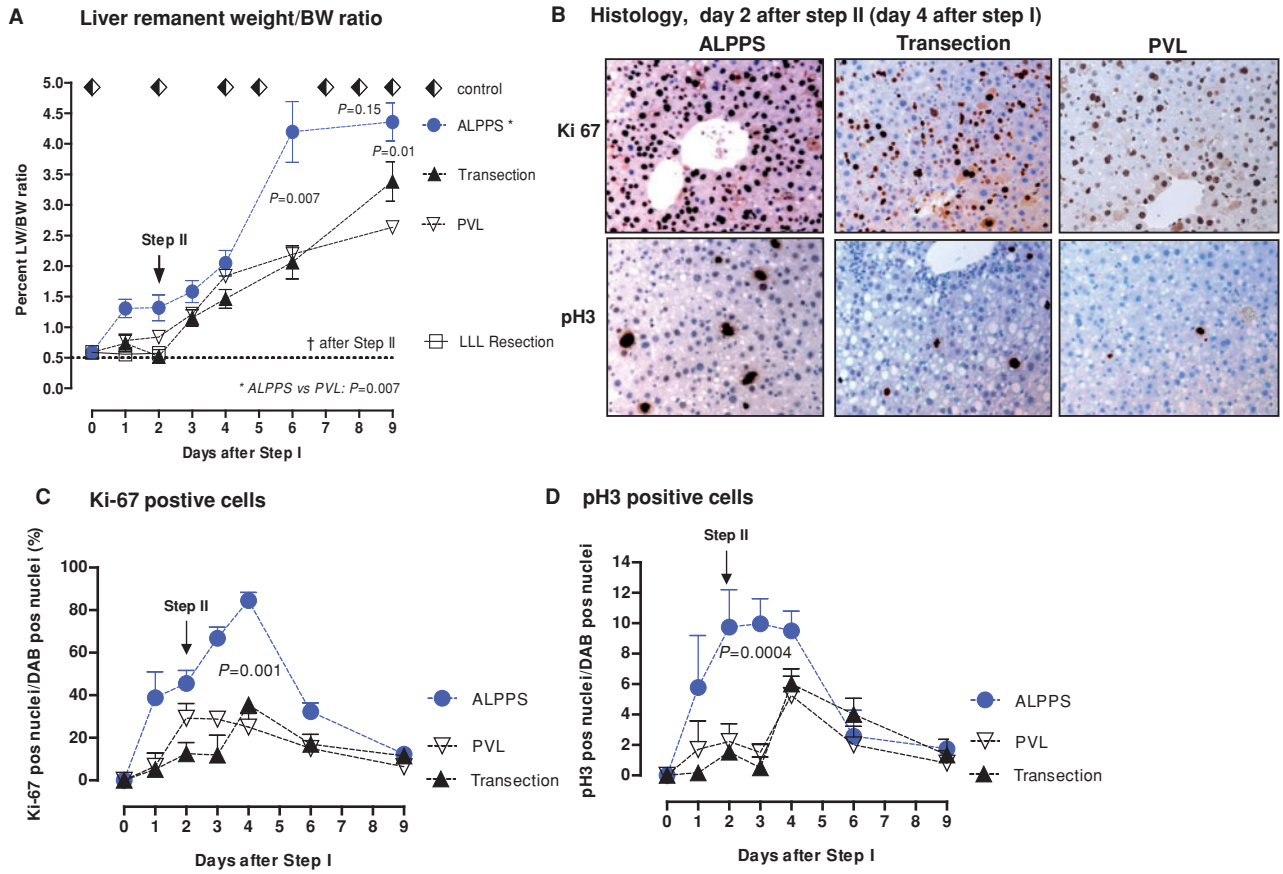

FIGURE 4. Volume gain and regeneration markers after completion of ALPPS procedure—step I and II. A, Liver volume combining steps I and II in all different groups. Two days after step I, we performed step II. The peak of regeneration was detected at 4 days after step II, whereas ALPPS mice displayed an accelerated hypertrophy as compared with mice with PVL or transection alone [LR (liver remnant)/BW ratio: ALPPS vs PVL: day 4: $P = 0.007$, day 7: $P = 0.007$; ALPPS vs transection: day 7: $P = 0.01$)]. Following 1 week of observation after step II, LR after ALPPS terminated growth when basically a new liver was grown derived from initially 10% FLR. Following step II surgery, mice allocated to the ALPPS group showed significant more hepatocytes, which entered the cell cycle, when compared to transection or PVL alone (B to D). BW indicates body weight; LW, liver remanent weight. †indicates animals after step II, two days after LLL resection, which where euthanized due to insufficient liver remanent weight.

of "cross portal" circulation after transection between the normally perfused and deportalized liver parts.[10,11] We have speculated that transection of the parenchyma may induce an inflammatory response with the release of putative growth factors, which may enhance the regenerative process. A few sets of experiments in the model of ALPPS strongly point out to a release of proliferating factors into the systemic circulation, which, combined with PVL, induce the unprecedented growth of the FLR. The observation of accelerated regeneration, when injecting plasma obtained from mice after ALPPS step I to animals undergoing PVL alone strongly supports the existence of soluble growth factors. The similar effects on regeneration achieved by injuries to other organs further point out to existence of soluble mediators of liver regeneration, additionally suggesting that the origin of the circulating growth factors is not "liver-specific." Of relevance was the detection of similar enhanced release of proinflammatory cytokines in our samples obtained in ALPPS and PVL patients. Despite the small sample size of patients, the dramatic increased expression of early instigators of regeneration (eg, IL-6 and TNF-α) after ALPPS, but not after PVL alone, was consistent with the observation in the rodent model of ALPPS. These findings open the door for future research to identify the responsible molecules triggering the accelerated regeneration.

The consistently reported higher morbidity after ALPPS,[6,10,13,14,20,23] including the development of multiorgan failure, has discouraged many groups to use this approach. With the observation of massive release of inflammatory factors into the circulation, it is tempting to speculate that these mediators may be responsible for the systemic inflammatory response syndrome, also known as SIRS or SIRS-like syndrome,[24,25] observed in several patients after ALPPS. Surgical procedures of similar invasiveness, that is, split liver in liver transplantation or living liver donation, which also include a complete liver transection, were shown to induce comparable systemic inflammation and cytokine release in patients.[26] We detected both in mice and in humans unusual increased levels of auxiliary promoters of liver regeneration, such as *IL-6 and TNF-α,* in the plasma[27,28] and in the liver tissue early after step 1 of ALPPS. Such molecules are known to contribute to SIRS-like syndrome and potentially enhance higher morbidity.[29,30] In human, however, SIRS occurred only after step II, whereas we investigated the release of inflammatory mediators only after step I for the search

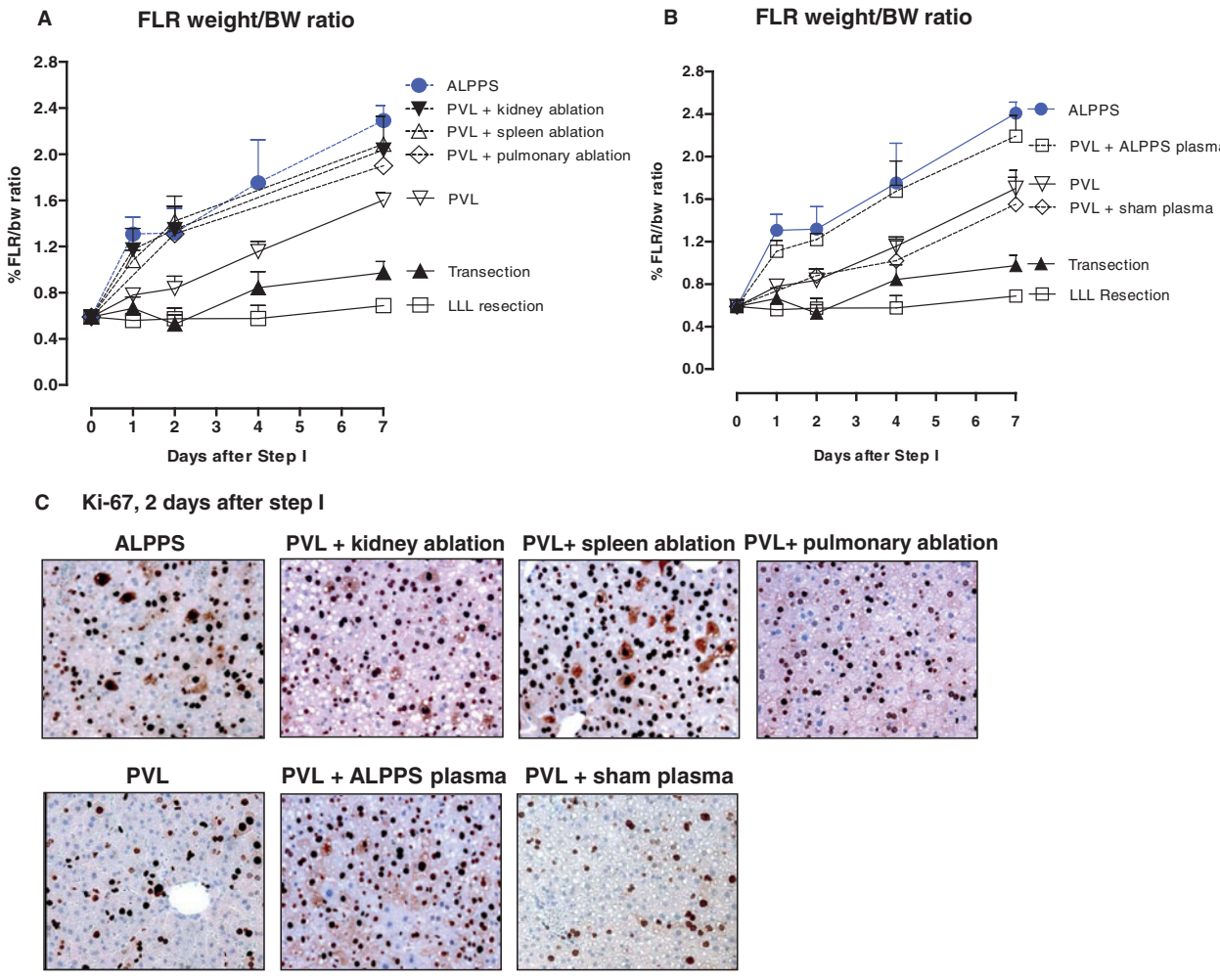

FIGURE 5. Volume gain and proliferation markers in future liver remnants during the first week after PVL combined with ablation of distant organs or injection of ALPPS plasma. Renal, splenic, or pulmonary ablation (A) or ALPPS plasma injection (B) together with PVL resulted in a comparable FLR volume gain as the original ALPPS procedure. Replacement of liver parenchyma transection by distant organ ablation or ALPPS plasma injection displayed the same number of proliferating hepatocytes, as shown by Ki-67 staining (C). BW indicates body weight.

of mechanisms of regeneration. Whether those mediators are still present after step II and how they may influence outcomes need further investigations.

The discrimination between molecules that induce liver regeneration from those, which may contribute to the increased morbidity, might be crucial to improve outcome after the ALPPS procedure, while preserving regeneration. Better knowledge on the timing of release may also be decisive for future therapeutic interventions.

Any animal model yields some limitations.[31] One difference between the ALPPS, as performed in the clinic and this mouse model, lies in size and lobular architecture of the liver. We carefully adapted, however, our mouse model to mimic the clinical situation. We removed the LLL lobe to reduce the lobulated liver shape and achieved 90% ligation of the portal vein branches, followed by liver transection. This approach led to a model comparable with ALPPS in humans, and controls were appropriately designed to secure a negligible impact of the resection of a small part of the liver during step I.

CONCLUSIONS

The availability of a mouse model of ALPPS, comparable with ALPPS performed in humans, enabled to confirm the accelerated liver regeneration associated with this procedure. The systemic release of circulating proliferating factors related to parenchymal transection in association with PVL seems to be crucial for the rapid and efficient liver growth. Future work may focus on the identification of the specific molecules, leading to the discovery of novel pathways of liver regeneration, and perhaps new targets to control the SIRS-associated morbidity, while enhancing the regenerative capacity of the liver.

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DISCUSSANTS

P. Lodge (Leeds, United Kingdom):

ALPPS, the combination of associating liver partition with portal vein ligation for staged hepatectomy, is clearly very topical, and there has been much discussion in the past 3 years in the major liver meetings primarily related to indications and safety and also about the mechanisms related to the rapid future liver remnant growth that occurs. This important work goes some way to answering these questions. Thank you for allowing me to review the full article before this meeting. This study represents an enormous volume of work, amounting to experimental surgery on 563 mice, which translates into more than 1000 surgical procedures. The experimental model was complex and you should be congratulated on its results.

Questions:

[1] One interesting aspect is that distant organ damage, in combination with portal vein ligation, produced future liver remnant growth that seemed to be as rapid as it is with ALPPS. In addition, portal vein ligation mice treated with plasma achieved equally rapid liver hypertrophy as ALPPS mice did. This seems to relate to putative growth factors associated with tissue damage. As this has not really been seen to such an extent in 2-stage liver surgery, in which a right portal vein ligation is mostly combined with a left liver resection or an initial ablation, do you think that the amount of tissue destruction, associated with liver transection in ALPPS, is greater because, in reality, an ALPPS segment 4 is completely devascularized, as it loses both its portal vein and arterial supply? Perhaps, could it be that the degree of tissue destruction is relevant to ALPPS?

[2] In the human aspect of your study, and in the mice, you measured IL-6 and TNF-α, which showed a rapid rise after the first stage of ALPPS. CRP is often used as a measure of the systemic inflammatory response after surgery. In many types of surgery, we are not keen to see a rise in CRP, but after major liver resection, an early rise seems to be associated with a more rapid recovery, perhaps related to faster liver regeneration. Did you consider including CRP in your study and have you looked at this clinically in your ALPPS patients?

Once again, you should be congratulated on an impressive study, which represents an enormous body of work. I would like to thank the ESA for the opportunity to review and comment on this article.

Response From A. Schlegel (Zurich, Switzerland):

Thank you for your compliments and questions. I would like to start with your second question about CRP. In animals, CRP is a poor marker of inflammation and any specific type of injury and therefore, cannot be used reliably. In the clinical setting of ALPPS, we did not observe significant differences in CRP levels in patients, when compared with those, who underwent portal vein ligation.

Your first question deals with the possible role of segment IV devascularisation inducing cytokine release and liver regeneration.

Our experimental design does not address this issue. However, enhanced regeneration occurs without devascularisation of a liver segment. In this setting, our experiments indeed indicate that a minimal degree of injury is required to trigger regeneration in the liver.

DISCUSSANTS

C. Bruns (Magdeburg, Germany):

Thank you very much for this nice, experimental article. I just have 1 brief question. Through your experiments on mice, you showed that Ki-67 increased after ALPPS and was, thus, a marker for proliferation. First of all, those livers were completely healthy, without having been exposed to chemotherapy. This represents a problem in your model.

You then also looked at the enlarged liver lobes, with respect to vascularization. Did you perform H&E staining or immunohistochemical staining, when analyzing whether enlarged liver lobes increase blood flow associated with angiogenesis?

Response From A. Schlegel (Zurich, Switzerland):

Thank you, Professor Bruns, for your question. We agree that the mice livers were healthy, but this actually represents an advantage of such models, enabling us to assess a specific question in a standardized manner.

We performed immunohistochemistry, that is, CD 31 staining, which targeted endothelial cells and could not detect differences, regarding intrahepatic microvascular density or angiogenesis in the regenerating lobe. In addition, we quantified the vascular endothelial growth factor (VEGF) expression and observed no differences during the first week after ALPPS or only portal vein ligation. Further research will focus on underlying mechanisms of regeneration, including endothelial cells and other nonparenchymal cells.