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Lymphocytes Support Oval Cell-Dependent Liver Regeneration

Hélène Strick-Marchand,†* Guillemette X. Masse,†* Mary C. Weiss,‡§ and James P. Di Santo2*†

In case of hepatic damage, the liver uses a unique regeneration mechanism through proliferation of hepatocytes. If this process is inhibited, bipotent oval stem cells proliferate and differentiate to hepatocytes and bile ducts, thus restoring liver mass. Although oval cell accumulation in the liver is often associated with inflammatory processes, the role of lymphocytes in oval cell-mediated hepatic regeneration is poorly understood. We treated wild-type and immunodeficient mice with an oval cell-inducing diet: in the absence of T cells (CD3ε/−/− and Rag2−/−) there were fewer oval cells, whereas in alymphoid mice (Rag2−/−γc−/−) a strongly reduced oval cell response and higher mortality, due to liver failure, was observed. Adoptive transfer of T cells into alymphoid mice protected them from liver failure, but was insufficient to restore the oval cell response. Treatment of Rag2−/−/H11546 mice with an NK cell-depleting Ab resulted in a significantly diminished oval cell response. These genetic experiments point to a major role for NK and T cells in oval cell expansion. In wild-type mice, oval cell proliferation is accompanied by an intrahepatic inflammatory response, characterized by the recruitment of Kupffer, NK, NKT, and T cells. Under these conditions, lymphocytes produce Th1 proinflammatory cytokines (IFN-γ and TNF-α) that are mitogenic for oval cells. Our data suggest that T and NK lymphocytes stimulate oval cell expansion by local cytokine secretion. This beneficial cross-talk between the immune system and liver stem cells operates under noninfectious conditions and could promote tissue regeneration following acute liver damage. The Journal of Immunology, 2008, 181: 2764–2771.

The primary functions of the liver are to regulate glucose, lipid, and amino acid metabolism, although this organ also serves to remove pathogens, dietary Ags, and xenobiotics from the circulation. The local immune response in the liver appears tightly regulated because it participates in the elimination of pathogenic microorganisms while helping to maintain tolerance to orally ingested Ags.

In the liver, Ags are presented by Kupffer cells, dendritic cells, and sinusoidal endothelial cells; the latter line the vasculature and inhibit direct contact between circulating leukocytes and hepatocytes (1). Intrahepatic leukocytes encompass 25% of liver cells, among which resident macrophages (Kupffer cells), NK cells, NK T cells, and CD4+ and CD8+ T lymphocytes are highly represented (2, 3). Intrahepatic lymphocytes (including NK, NK T, and some T cell subsets) are activated following recognition of modified self-Ags that reveal cellular changes such as viral infection, transformation, changes in MHC class I expression, and stress-induced proteins (4, 5).

A “cross-talk” between the immune system and the liver has been observed in human diseases such as fulminant and chronic hepatitis in which liver regeneration is accompanied by an inflammatory reaction. In this context, inflammation may be responsible for tissue damage, although in other situations it may be involved in the regulation of tissue repair (6). For example, fibrosis is enhanced by macrophages that secrete TGF-β1 and activate hepatic stellate cells to produce collagen I (7); hepatic NK cells regulate fibrosis by killing activated stellate cells in an NKG2D-dependent manner (8). Lymphocytes are recruited to the liver during human diseases by cholangiocytes through chemokine-receptor interactions (such as SDF-1/CXCR4, fractalkine/CX3CR1, and CXCL16/CXCR6) (9–12). It is not always clear whether lymphocyte recruitment plays a beneficial (protective) or detrimental (destructive) role under these conditions.

Liver regeneration is accomplished through hepatocyte and cholangiocyte proliferation, which restores the damaged tissue. However, if hepatocyte proliferation is inhibited, liver repair is undertaken by hepatic progenitor cells (oval cells), which proliferate, migrate, and differentiate into hepatocytes and cholangiocytes (13, 14). The mechanisms that regulate these mutually exclusive pathways of regeneration are not fully understood.

Quiescent oval cells are localized at the junction between hepatocytes and cholangiocytes in the canals of Hering. They are characterized by an ovoid nucleus, scant cytoplasm, and the expression of fetal hepatocyte (α-fetoprotein and cytochromes CK8 and CK18) (15, 16), cholangiocyte (A6 and cytokeratins CK7 and CK19) (14), and hematopoietic stem cell markers (CD90, CD117, CD34, Sca-1, and CD45) (17–20). In humans, proliferating oval cells are observed during chronic liver diseases such as cholestasis, hepatitis B, and hepatitis C (21–23). Deciphering the mechanisms that promote oval cell expansion is essential to understanding liver regeneration, particularly during chronic liver diseases.

Molecular pathways regulating oval cell proliferation include soluble factors, secreted during inflammatory reactions, such as IFN-γ, IFN-α, TNF-α, lymphotoxin-β, IL-15, TWEAK, and...
TGF-β (24–29). Furthermore, oval cells express the receptors for these cytokines, and the inactivation of some of these signaling pathways reduces (but does not eliminate) their proliferation in vivo, suggesting functional redundancy (26, 30–33). The cells responsible for this cytokine production, which leads to oval cell expansion in vivo, have not been clearly defined. It has not been addressed whether the intrahepatic inflammation, observed during oval cell-mediated liver regeneration, leads to tissue damage or repair.

Considering these observations, we evaluated the roles of different intrahepatic lymphocytes during oval cell expansion following hepatic stress. The choline-deficient ethionine-supplemented (CDE) diet induces a strong oval cell response in normal mice. We assessed the oval cell response in livers of μMT−/− mice deficient in B lymphocytes (34), CD3ε−/− mice devoid of T lymphocytes (35), Rag2−/− mice lacking B and T lymphocytes (36), Rag2−/− mice treated with an NK cell-depleting Ab, Rag2 and common γ chain (γc)-deficient mice devoid of B cells, T cells, and NK cells (Rag2−/− γc−/− mice) (37), and Rag2−/− γc−/− mice adoptively transferred with polyclonal αβ T cells. Our results demonstrate that alymphoid mice die of acute liver failure and are unable to mount an adequate oval cell response. Mice harboring either NK or T cells survive; however, both T and NK cells are required for mice to develop a strong oval cell response. Collectively, our data strongly suggests that intrahepatic lymphocytes support oval stem cell expansion, presumably through their cytokine production, thereby revealing a beneficial cross-talk between the regenerating liver and the immune system.

Materials and Methods

Animals and CDE diet

All mice were on the C57BL/6 background. The 5- to 6-wk-old wild-type (WT), μMT−/− (34), CD1d−/− (38), CD3ε−/− (35), Rag2−/− (36), and Rag2−/− γc−/− (37) male mice were fed either choline-deficient modified chow (ICN) and 0.165% DL-ethionine (Acros Organics) in the drinking water or standard laboratory rodent chow and water. In some experiments, NK cells were depleted in Rag2−/− mice by i.p. administration of purified anti-NK1.1 Ab (clone PKI36, 100 µg each week for 3 wk; BD Pharmingen). Complete depletion of NK cells was verified by FACS analysis. Enriched T cells from lymph nodes of C57BL/6 mice were adoptively transferred to 4-wk-old Rag2−/− γc−/− mice as described (39). Three weeks after the transfer, the mice began treatment with the CDE diet. After 1 wk of CDE diet, mice were bled and assayed for lymphocyte reconstitution by FACS, and serum content for liver enzymes. Serum was analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and total bilirubin using an Olympus AU400 analyzer with kits and controls supplied by Olympus. Animals received humane care and were kept under pathogen free conditions in accordance with institutional guidelines.

Histological techniques

Immunohistochemistry of cryostat sections was performed as previously described (40). For immunofluorescence staining, the slides were prepared as for immunohistochemistry, but the Abs were diluted in PBS containing 0.1% BSA, 0.1% Triton X-100 and 1% FCS. The following Abs were used: A6, which was a gift from N. V. Engelhardt (Institute of Carcinogenesis, Moscow, Russia), F4/80 (clone CI:A3-1; Serotec), CD4 (clone H129.19; BD Pharmingen), NKp46/NCR1 (BAF2225; R&D Systems), Ki67 (VP-RM04; Vector Laboratories), goat anti-rat-HRP (CalTAG Laboratories), donkey anti-rat Alexa Fluor 488 and donkey anti-goat Alexa Fluor 555 (both from Molecular Probes). For A6 quantification, five photographs at a magnification of ×100 were analyzed per liver. The percentage of A6+ tissue was determined by multiplying the number of A6 stained pixels by 100 and dividing it by the total number of pixels per photograph using Adobe Photoshop CS (Adobe Systems).

Abbreviations used in this paper: CDE, choline-deficient ethionine-supplemented; γc, common γ chain; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; WT, wild type.

Flow cytometric analysis

Each experiment, consisting of one control and two CDE diet-treated mice, was repeated two to four times. Mice were sacrificed, and the liver perfused with PBS to eliminate circulating leukocytes. Liver leukocytes were purified by centrifugation on an 80/40% Percoll gradient, and remaining erythrocytes were lysed. The mAbs used were conjugated to FITC, PE, PE-Cy5.5, PerCP-Cy5.5, PE-Cy7, allophycocyanin-Cy7, or allophycocyanin-Cy5.5 (BD Biosciences), and ionomycin (1 µg/ml; Sigma-Aldrich). Cells were stained for extracellular markers, fixed with Cytofix/Cytoperm solution (BD Biosciences), permeabilized with Perm/Wash solution (BD Biosciences), and incubated with anti-IFN-γ-PE (XM1G1.2; BD Pharmingen)

FIGURE 1. Liver regeneration mediated by oval cells is severely impaired in alymphoid mice. Immunohistochemistry, using A6 Ab, of liver sections from lymphocyte-deficient mice fed a control diet (left column) or a CDE diet for 3 wk (right column). A6 stains bile duct (BD) structures and oval cells migrating from the portal tract (PV) into the liver parenchyma. Magnification is ×100.
or anti-TNF-α-FITC (MP6-XT22; eBioscence). FACS acquisition was performed using a FACSCalibur or a FACScanto analytical flow cytometer (BD Biosciences), and data were analyzed using FlowJo 6.4. Gates were set on isotype controls. The percentage of immunostained cells determined from the FACS data and the total liver leukocyte counts enabled us to calculate the number of cells in each population. Statistical analysis was performed using the Mann-Whitney U test on all the data.

Results

Oval cells do not expand in alymphoid Rag2−/−γc−/− mice

Following previous observations that oval cell proliferation is accompanied by an inflammatory response in the liver (41), we wished to determine the role played by lymphocytes in oval cell-mediated liver regeneration. Hepatic architecture was studied in WT mice and mice genetically deficient for B cells (μMT−/−), T cells (CD3e−/−), B and T cells (Rag2−/−), and B cells, T cells, and NK cells (Rag2−/−γc−/−) (all on the C57BL/6 background). No differences were observed in terms of gross liver morphology and liver histology when these mice were fed a normal chow diet (Fig. 1). In all cases the A6 marker (revealing cholangiocytes and oval cells) was normally expressed and revealed a single-cell layered A6+ bile duct formed by cholangiocytes, in the vicinity of the portal vein and artery, composing a normal portal triad (Fig. 1).

To induce liver regeneration via oval cell proliferation, we treated mice with a CDE diet for 3 wk (42). This diet is cytotoxic to hepatocytes, causing severe liver damage via steatosis within a week and the induction of oval cell proliferation to regenerate the tissue within 2 wk. The most abundant oval cells, identified by their expression of A6 (or cytokeratin CK19), are observed after 3 wk near bile ducts and migrating between hepatocytes. WT and μMT−/− mice, treated with the CDE diet for 3 wk, had similar numbers of A6+ oval cells, indicating that B lymphocytes were not essential for this process (Fig. 1). CD1d−/− mice had an oval cell response comparable to that of WT mice, suggesting that NKT cells were not essential for this process (data not shown). In contrast, following CDE treatment, we observed a strikingly higher mortality rate in Rag2−/−γc−/− mice (58%) (p = 0.0003) and Rag2−/− mice (25%) compared with WT mice (<1%) after 3 wk of treatment.

We analyzed the serum of WT and Rag2−/−γc−/− mice after 1 wk of treatment: Rag2−/−γc−/− mice have very elevated levels of ALT, AST, total bilirubin, and LDH after 1 wk of CDE diet, compared with WT mice (Fig. 2). The serum from Rag2−/−γc−/− mice also shows that the mice are severely jaundiced. These high levels of liver enzymes indicate that the CDE diet is more hepatotoxic in the alymphoid mice and causes severe liver damage, which accounts for the high mortality rate observed in this group.

We assessed whether the oval cell response was modified in the different genotypes by quantifying A6+ cells from multiple fields for each liver (Fig. 3). The ductular reaction was reduced and fewer A6+ cells were present in the CD3e−/−, Rag2−/−, and Rag2−/−γc−/− mice (Figs. 1 and 3), thereby implicating a role for T cells, NK cells, or both in oval cell expansion. Whereas the CDE-treated WT mouse contains a mean of 9.7% A6+ cells at 3 wk, CD3e−/−- or Rag2−/−-treated mice contained 7.3% and 6.6% A6+ cells, respectively. A total of 4 of 22 WT mice showed high levels of oval cells (18–24%), whereas similar levels were observed in only 1 of 16 CD3e−/− mice and in only 1 of 11 Rag2−/− mice (Fig. 3). A significantly diminished oval cell response was observed with alymphoid Rag2−/−γc−/− mice in which only 4.5% of the liver was composed of A6+ oval cells (p = 0.003).

To determine whether this strongly reduced oval cell response was due to the lack of NK cells, or whether it was due to the lack of signaling through the γc chain (which is the receptor for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21), we treated NK cell-depleted Rag2−/− mice with the CDE diet for 3 wk. Complete NK cell depletion was verified by FACS analysis of the spleen and liver (data not shown). These mice had a significantly reduced oval cell response, similar to the Rag2−/−γc−/− mice (Fig. 3) (p = 0.01), suggesting that γc-dependent cytokines are not involved in the oval cell response but rather that the NK cells per se play an important role in this process.

We next determined whether adoptive transfer of T cells into Rag2−/−γc−/− mice could rescue the hepatic phenotype. The
Rag2\(^{-/-}\)\(\gamma_c^{-/-}\) mice reconstituted with T cells treated with CDE had a better survival rate and slightly elevated levels of liver enzymes (ALT, AST, total bilirubin, LDH) (Fig. 2). After 3 wk of CDE diet, the Rag2\(^{-/-}\)\(\gamma_c^{-/-}\) mice adoptively transferred with T cells were analyzed for the presence of oval cells. Interestingly, we did not observe a significant oval cell expansion in these mice (Fig. 3). Therefore, although T lymphocytes were sufficient to protect the liver from the increased cytotoxicity observed in lymphoid mice, they were insufficient to induce an oval cell response.

To clarify whether lymphocytes are required for oval cell proliferation, we quantified double positive A6 and Ki67 cells using immunofluorescence staining. Although there was a severely reduced percentage of oval cells in Rag2\(^{-/-}\) and Rag2\(^{-/-}\)\(\gamma_c^{-/-}\) mice, there was no significant difference in the percentage of proliferating oval cells in the immunodeficient compared with the WT mice, indicating that the few oval cells present in these immunodeficient mice could proliferate (data not shown).

**NK and CD4\(^+\) T lymphocytes colocalize with oval cells in the liver of CDE-treated mice**

In a WT liver at steady-state conditions, rare NK cells and CD4\(^+\) and CD8\(^+\) T lymphocytes are observed near the portal vein and between hepatocytes, without a particular zonal distribution within the liver lobule (data not shown and Fig. 4). However, following the CDE treatment, the inflammatory response is concentrated around the portal vein. To determine the positions of intrahepatic lymphocytes relative to oval cells, we immunostained sections from CDE-treated mice using A6 and lymphocyte specific Abs. NK cells, recognized by their expression of NKp46, were adjacent both to bile ducts and to oval cells in WT and Rag2\(^{-/-}\) mice throughout the duration of treatment (Fig. 4A). Numerous Kupffer cells (F4/80\(^+\)) were observed in the liver parenchyma, accumulating between hepatocytes and neighboring oval cells without a specific location (Fig. 4B). CD4\(^+\) T lymphocytes were clustered in close proximity to oval cells near the portal vein (Fig. 4B). CD8\(^+\) T cells were grouped around bile ducts and were not observed near migrating oval cells (Fig. 4B). Thus, during the inflammatory response to the CDE diet, intrahepatic NK and CD4\(^+\) T lymphocytes colocalize with oval cells, whereas CD8\(^+\) T cells cluster around the bile ducts from which oval cells are thought to emerge.

**The regenerating liver is enriched in Kupffer cells, activated NK cells, NKT cells, and mature CD4\(^+\) and CD8\(^+\) T lymphocytes**

Our observations suggest that NK and T lymphocytes are involved in oval cell expansion. To precisely quantify each leukocyte population and determine the state of activation of specific lymphocyte populations, we characterized the inflammatory infiltrate in WT livers from control and CDE-treated mice (at 2 and 3 wk posttreatment), using flow cytometry. Livers from mice having received a CDE diet for 2 or 3 wk contained more leukocytes than controls (1.5-fold). Macrophages and neutrophils are the first hematopoietic cells that may be recruited to inflammatory sites. An increase in the percentage and total number of macrophages (CD11b\(^+\)Gr1\(^{low}\)) was observed after 2 wk of CDE diet, which persisted at 3 wk, whereas neutrophil (CD11b\(^+\)Gr1\(^{high}\)) frequency and number did not change significantly (Fig. 5). The latter was expected because enhanced neutrophil influx often accompanies infectious processes. No differences were seen in the number of B lymphocytes (CD19\(^+\)IgM\(^+\) or CD19\(^+\)IgD\(^+\)) or dendritic cells (data not shown). There was no significant change in the composition of spleen leukocytes, suggesting that the CDE diet induced a local (noninfectious) inflammatory response in the liver.

Innate lymphocytes patrol the liver sinusoids and include NK cells and NKT cells. We observed a significant increase in the percentage and number of mature NK cells (DX5\(^+\)NK1.1\(^+\)) in the liver following 2 wk of CDE diet, which declined slightly at 3 wk (Fig. 5). Importantly, more NK cells showed an activated phenotype (CD69\(^+\)) in the context of oval cell proliferation (Fig. 5). Furthermore, a rise in the percentage and number of NKT cells (NK1.1\(^+\)CD3\(^+\)) in the liver was observed after 3 wk of the diet, although these cells were not acutely activated (Fig. 5).

T lymphocytes are critical effector cells in the immune system. The percentage of CD4\(^+\) T cells (CD3\(^+\)CD4\(^+\)) was decreased following the diet, yet their absolute number did not change significantly (Fig. 5). Within the CD4\(^+\) T population, a higher percentage of cells expressed the activation marker CD44 after 3 wk of

**FIGURE 4.** NK and T cells colocalize with oval cells and bile ducts in the regenerating liver. A, Liver sections from WT and Rag2\(^{-/-}\) mice treated with a control or CDE diet for 2 or 3 wk, analyzed by immunofluorescence using the A6 Ab (green) to recognize oval cells, and the NKp46 Ab (red) to visualize NK cells. Portal vein (PV) and bile duct (BD) are shown. Magnification is at ×200. B, Immunohistochemical analysis of serial liver sections of WT mice treated with CDE for 3 wk. F4/80 recognizes macrophages, and CD4 and CD8 reveal each subtype of T lymphocyte. Dashed ovals show regions of interest as described in Results. Magnification is at ×200.
CDE, although their absolute number was not modified. The percentage and number of CD8+/H11001 T cells (CD3+/H11001 CD8+/H11001) did not vary; however, more of these cells displayed an activated phenotype (CD44+/H11001) (Fig. 5). Both the CD3+/H11001 CD4+/H11001 CD44+/H11001 and the CD3+/H11001 CD8+/H11001 CD44+/H11001 cells were NK1.1+/H11002, confirming that these are not a subset of NKT cells. These results show that the CDE treatment induces an activation of T lymphocytes in the liver.

The oval cell response in T cell-deficient mice is accompanied by the activation of NK cells

Because T lymphocyte-deficient mice still retain a moderate oval cell reaction (CD3+/H9255/CD8+/H11002 and Rag2+/H11002/CD8+/H11002 mice) (Figs. 1 and 3), we further characterized the inflammatory response in these mice. Comparison of control and CDE-treated CD3+/H9255/CD8+/H11002 and Rag2+/H11002/CD8+/H11002 mice showed no significant change in the absolute number of macrophages, yet the NK cell number was somewhat increased following the treatment (Fig. 6). More importantly, both CD3+/H9255/CD8+/H11002- and Rag2+/H11002/CD8+/H11002-treated mice demonstrated a significantly higher proportion of NK cells with an activated phenotype (NK1.1+/DX5+/CD69+) compared with nontreated mice (Fig. 6).

Cytokine secretion by intrahepatic lymphocytes during the CDE treatment

We next determined whether increased hepatic infiltration of NK and NKT cells, and the mature phenotype of CD4+ and CD8+ T cell subsets, were associated with increased cytokine production. CD3e–/– and Rag2–/– mice treated with a CDE diet have a reduced inflammatory response mediated by NK cells. Dot plot shows the absolute number of intrahepatic cells for each population analyzed by FACS. Mature (NK1.1+DX5+) and activated NK (NK1.1+DX5+CD69+) cells were quantified from CD3e–/– mice (A) and Rag2–/– mice (B) subsequent to a control or CDE diet (for 3 wk). Each symbol represents a mouse. The horizontal bar represents the mean of the group. *, p < 0.01.
cells in CDE-treated mice, was associated with the activation of effector functions such as the production of IFN-γ and TNF-α. Intrahepatic lymphocytes were isolated from control or CDE-treated (10 days) WT mice, restimulated in vitro, and analyzed by FACS. At this early time point of oval cell expansion, NK and NKT cells from CDE-treated mice secreted significantly more IFN-γ and TNF-α than cells from control mice (Fig. 7, A and B).

To determine whether the production of cytokines by NK and NKT cells was maintained for the duration of the oval cell response, we analyzed mice treated with CDE for 3 wk. Without restimulation in vitro, we observed an increase in the number of NK and NKT cells that produce IFN-γ and TNF-α in mice treated with CDE compared with controls (Fig. 7C). The analysis of CD4⁺ and CD8⁺ T cells revealed a similar pattern, whereby a
significantly higher proportion of cells from CDE-treated mice produced IFN-γ and TNF-α at the onset of oval cell expansion (10 days) (Fig. 8, A and B), as well as at later time points (3 wk) (Fig. 8C), compared with mice on a control diet. Together, these results show that concomitantly to oval cell expansion, lymphocytes are primed to secrete proinflammatory type 1 cytokines including IFN-γ and TNF-α, and that this inflammatory response continues throughout oval cell-mediated liver regeneration.

Discussion

The signaling pathways and molecular mechanisms that regulate liver regeneration via hepatocyte proliferation or by oval cell expansion are only partially understood. Previous studies showed the mitogenic potential of inflammatory cytokines (IFN-γ, TNF-α, lymphokinin-β, IL-15) for oval cells (24–26, 33). As oval cell-mediated liver regeneration is accompanied by an inflammatory response, these cytokines could play an important role. Inhibiting this response, by treating with dexamethasone or a cyclooxygenase 2 inhibitor, reduces the expansion of oval cells in the damaged liver (43, 44).

We have determined which leukocyte subsets are involved in liver repair using a model that mimics severe hepatic stress. We show that NK and T cells are required for an optimal oval cell expansion in mice receiving a CDE diet: T cell-deficient mice harboring NK cells (CD3ε−/−, Rag2−/−) show few oval cells, the depletion of NK cells from Rag2−/− mice further reduces the quantity of oval cells, aldehyde bodies (Rag2−/−/γ−/−) are essentially devoid of an oval cell response and have a high mortality rate due to liver failure, and aldehyde bodies reconstituted with T cells are protected from liver failure yet do not mount a substantial oval cell response. Together, these results suggest that both NK and T cells are required for oval cells to expand and regenerate the liver.

Our data reveal that intrahepatic NK cells and CD4+ T cells colocalize with expanding and migrating oval cells, whereas CD8+ T cells localize near bile ducts. Characterization of intrahepatic leukocytes from CDE-treated WT mice demonstrated an increase in total leukocytes, consisting of Kupffer cells, activated NK cells, NKT cells, and activated CD8+ T cells. Furthermore, we show that these lymphocytes produce significantly more Th1 proinflammatory cytokines IFN-γ and TNF-α. These results strongly suggest that activated lymphocytes provide critical soluble factors that stimulate oval cell expansion in the context of a CDE treatment.

Th17 T cells are a recently described subset of differentiated CD4+ T cells that produce IL-17 and are implicated in several inflammatory conditions (45). Our preliminary data indicate that treatment of RORγt-GFP mice (that can be used to identify Th17 T cells (46)) with the CDE diet resulted in a 2-fold increase in the percentage of GFP+ T cells in intrahepatic infiltrates (data not shown). These results suggest that the Th17 pathway may also be activated during oval cell expansion.

The similarly reduced oval cell response and NK cell activation observed in the T cell-deficient mice (CD3ε−/− and Rag2−/−) suggests that multiple signaling pathways are able to induce oval cell-mediated liver repair. Under conditions of severe stress, hepatocytes produce acute phase proteins and complement components that activate Kupffer cells (5). Mice treated with gadolinium chloride, which inhibits Kupffer cell activity, did not mount an efficient oval cell response (47). Furthermore, the CDE diet causes an accumulation of lipids, which becomes hepatotoxic. CD1-restricted NKT cells respond to lipid Ags and activate NK cells (48).

Our results show that the few oval cells present in aldehyde bodies are proliferating. One explanation for the high mortality rate seen in Rag2−/−γ−/− mice is that the remaining Kupffer cells initiate an oval cell response, yet these cells do not receive sufficient signals to expand to significant levels, perhaps due to the necessity of multiple and diverse inductive signals. Alternatively, a cytokine threshold may be required for the activation of oval cells and their optimal expansion. Thus, the few remaining oval cells seen in Rag2−/−γ−/− mice are insufficient to repair damage, leading to hepatic failure and death of the animal.

During muscle regeneration, it has been shown that macrophages contribute both to tissue damage, through the secretion of free radicals, and to tissue repair, through the production of growth factors and cytokines (49). In the case of the liver, lymphocytes have been shown to mediate hepatotoxicity: Con A treatment causes CD4+ T cell-dependent injury (50), primary biliary cirrhosis causes intrahepatic bile duct sclerosis in humans due in part to autoreactive T cells (51), and fibrosis is regulated by the balance between Th1 and Th2 cytokines (52). Liver regeneration is driven by an inflammatory reaction: hepatocyte proliferation is enhanced by cytokines such as TNF-α and IL-6 secreted by Kupffer cells, and C3a, C5a complement components (53, 54). However, depletion of NK cells before partial hepatectomy enhances hepatocyte proliferation yet does not modify liver regeneration in fine (55). Thus, a balance between inhibitory and inductive signals, mediated by the inflammatory reaction, regulates liver regeneration.

We propose the following model of interaction between oval cells and the immune system during CDE-mediated liver regeneration. The CDE diet is cytotoxic to hepatocytes, causing the release of cellular debris, and providing a danger signal that initiates an inflammatory response (56). Macrophages are recruited to the site of inflammation, activate and recruit innate NK and NKT cells, and adaptive CD4+ and CD8+ T cells. These lymphocytes, which colocalize with bile ducts, produce effector cytokines including IFN-γ and TNF-α, and thereby promote the expansion of oval cells. Although this immune reaction could be considered as a prototypical Th1 response, it is important to remember that it occurs under noninfectious conditions. As such, lymphocytes are essential for this nonimmune response that impacts on hepatic regeneration. This beneficial immune cross-talk could likewise influence repair and regeneration at other tissue sites.

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Disclosures

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