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Galectin-9: Diverse Roles in Hepatic Immune Homeostasis and Inflammation

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Abstract

Glycan-binding proteins, which include galectins, are involved at all stages of immunity and inflammation, from initiation through resolution. Galectin-9 (Gal-9) is highly expressed in the liver and has a wide variety of biological functions in innate and adaptive immunity that are instrumental in the maintenance of hepatic homeostasis. In the setting of viral hepatitis, increased expression of Gal-9 drives the expansion of regulatory T cells and contraction of effector T cells, thereby favoring viral persistence. The dichotomous nature of Gal-9 is evident in hepatocellular carcinoma, where loss of expression in hepatocytes promotes tumor growth and metastasis, whereas overexpression by Kupffer cells and endothelial cells inhibits the antitumor immune response. In nonalcoholic fatty liver disease, Gal-9 is involved indirectly in the expansion of protective natural killer T-cell populations. In ischemic liver injury, hepatocyte-derived Gal-9 is both diagnostic and cytoprotective. In drug-induced acute liver failure, plasma levels correlate with outcome. Here, we offer a synthesis of recent and emerging findings on Gal-9 in the regulation of hepatic inflammation. Ongoing studies are warranted to better elucidate the pathophysiology of hepatic immune-mediated diseases and to develop new therapeutic interventions using glycan-binding proteins.

Glycobiology is the study of the structure and function of glycans (i.e., carbohydrates, saccharides, or simple sugars). Because they are dominantly expressed on all eukaryotic and prokaryotic cell surfaces, glycans play central roles in cell–cell and cell–pathogen interactions.[(1)] Glycan-binding proteins are involved at all stages of immunity and inflammation, from initiation through resolution. In humans, more than 80 glycan-binding proteins have been described that fall into about a dozen structural families, each of which has a conserved carbohydrate recognition domain (CRD).[(1)] Galectins are a fascinating family of guanosine diphosphates that are secreted into the extracellular environment directly from the cytoplasm independently of the classical endoplasmic reticulum/Golgi trafficking machinery.[(2)] Galectins are evolutionarily expressed from nematodes to humans.[(3)] The prototype galectins have one CRD (e.g., galectin-1 [Gal-1]), whereas tandem repeat-type galectins contain two CRDs with differential preferences for carbohydrate

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binding separated by a linker (e.g., Gal-9), and the chimera-type (Gal-3) contains a single CRD connected to a nonlectin amino-terminal.[4] Galectins are expressed by many immune cells, including macrophages, dendritic cells, B cells, and T cells, as well as endothelial cells and stromal cells in many tissues, including abundant expression in the liver.[5,6] Gal-9 (LGALS9 in human, lgals9 in mouse) was first described as an eosinophil chemoattractant produced by T lymphocytes[7] and demonstrates important pleiotropic immune-regulatory properties.[2,5] Receptors or surface binding partners that have been reported for Gal-9 include T-cell immunoglobulin and mucin domain-containing molecule 3 (TIM-3),[8] glucose transporter 2, glucagon receptor, protein disulfide isomerase, Epstein-Barr virus latent membrane protein-1, immunoglobulin E, and the adhesion molecule cluster of differentiation 44 (CD44), which competes for hyaluronic acid.[9] The paradoxical activities of Gal-9 triggering T-cell death while activating innate immune cells such as dendritic cells to produce tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6; and greater phosphorylation of p38 and AKT) appear largely to be related to the N-terminal and C-terminal CRDs.[10,11] This review summarizes recent findings and describes emerging roles for Gal-9 in hepatic homeostasis and inflammation.

Specific Liver Diseases

VIRAL HEPATITIS

The most striking feature of hepatitis C virus (HCV) infection is its high propensity to establish chronicity, and the transition from acute to chronic infection is characterized by attenuated effector function and expansion of regulatory T cells.[12] We became intrigued with Gal-9 in HCV infection because of the findings that TIM-3 (the best studied natural ligand of Gal-9), a transmembrane protein with a stalk that anchors to an intracellular tail with an SH2 phosphorylation domain, is significantly up-regulated in T cells (particularly virus-specific) of HCV-infected patients, and that TIM-3 blockade could reverse exhaustion and restore $CD4^+$ and $CD8^+$ T-cell function in chronic infection.[13,14] We found that plasma levels of Gal-9 were markedly up-regulated in patients with chronic HCV and that the liver Kupffer cells (KCs) had the highest staining.[15] Moreover, peripherally derived macrophages differentiated with macrophage colony-stimulating factor and stimulated with interferon-gamma (IFN-γ) strongly induce Gal-9 in HCV-infected patients compared to controls, whereas Gal-9 is not induced by lipopolysaccharide, IL-1β, or HCV core protein (Toll-like receptor 2 [TLR2] stimulator).[15] Exosomes released from HCV-infected hepatocytes induced Gal-9 in cultured monocytes, and circulating nonclassical $CD16^{pos}CD14^{pos}HLA\text{-}DR^{pos}$ monocytes have the highest Gal-9 protein levels in chronically infected patients.[16] Stimulation of whole peripheral blood mononuclear cells with recombinant Gal-9 consistently expanded $CD4^+CD25^+FoxP3^+$ $CD127^{low}$ regulatory T cells (Tregs) in both HCV patients and normal controls, an effect mostly mediated by transforming growth factor beta[15] involving increased phosphorylation of Smad-2/3 and extracellular signal–regulated kinases-1/2.[17] Furthermore, Gal-9 can be produced by Tregs, in particular the $CD39^{pos}$ subset, and can inhibit proliferation and IL-21 production by HCV-specific $CD4^+$ T cells.[18] Gal-9 treatment activates caspase-8, inducing apoptosis of HCV-specific $CD8^+$ T cells (cytotoxic T lymphocytes),[15] which can be reversed with IL-21 derived from T helper 17 (Th17) cells.[18] In mice, Gal-9 suppresses generation of Th17

cells and IL-17 mRNA expression.[19] A paradigm for the roles of Gal-9 in HCV infection is presented in Fig. 1. Data on the role of Gal-9 in hepatitis B virus (HBV) infection are relatively sparse. As with HCV, KCs in HBV-infected liver biopsies express high levels of Gal-9, and serum levels of this protein are significantly increased compared to uninfected controls.[20,21] Moreover, TIM-3 expression is relatively increased on HBV-specific T cells and correlates with impaired effector function (IFN-γ production).[20]

AUTOIMMUNE HEPATITIS

Autoimmune hepatitis (AIH) is a progressive inflammatory condition characterized by hypergamma-globulinemia, circulating autoantibodies, and $CD4^+$ effector lymphocyte-mediated liver damage.[22–24] The percentage of $TIM\text{-}3^{pos}$ $CD4^+$ T-cell lymphocytes within the effector ($CD25^{neg}$) population is lower in patients with autoimmune liver disease (AILD; AIH and patients with primary sclerosing cholangitis overlap) and therefore less prone to immune suppression than in healthy controls.[22] Patients with AILD demonstrate lower percentages of circulating $Gal\text{-}9^{pos}$ cells within the $CD4^{pos}CD25^{pos}$ Treg subset than in healthy controls (Fig. 1). Moreover, the Gal-9 Treg subset in patients with AILD contains more proinflammatory IFN-γ^{pos} and $IL\text{-}17^{pos}$ cells and fewer transforming growth factor beta–positive and $IL\text{-}10^{pos}$ cells.[22] The observation of an inverse correlation between $Gal\text{-}9^{pos}$ Tregs and immunoglobulin G levels and titers of autoantibodies, which are the serological markers of the disease, further supports the concept that $Gal9^{pos}$ Tregs exert control over disease activity.[22] A significant proportion of patients with auto-immune liver disease fail to respond to conventional treatments, and these findings may have implications for Treg-based immunotherapy for AILD, including adoptive transfer of autologous, Gal-9 cultured cells or transfection of Tregs with Gal-9 complementary DNA.[22] In this regard, preclinical data in mice with concanavalin A–induced hepatitis,[25] a T cell–dependent model of liver damage, demonstrated disease exacerbation with TIM-3 blockade and improvement with administration of Gal-9.[25] Furthermore, Gal-9 in this model was also shown to inhibit production of the circulating proinflammatory cytokines TNF-α and IL-6. By increasing the Treg to T effector ratio, Gal-9 causes selective apoptosis of concanavalin A–activated $CD4^+$ T cells that further prevents the release of TNF-α, IL-6, and IFN-γ. Thus, inhibition of these important proinflammatory cytokines, which have cytotoxic effects on hepatocytes, represents an alternative mechanism for explaining the suppressive properties of Gal-9 and the potential therapeutic approach for T cell–mediated diseases.[25]

LIVER ISCHEMIC REPERFUSION INJURY

Liver ischemia/reperfusion injury (IRI) remains an important problem in clinical transplantation and after hepatic resection. Both innate and adaptive immunity have been implicated in mediating IRI.[26] For example, KCs trigger recruitment of $CD4^+$ T cells in the postischemic liver by releasing mediators, and $CD4^+$ T cells in turn influence the activation of KCs.[27] TIM-3/Gal-9 has been implicated in this crosstalk, as described in several elegant studies[27–30] (Fig. 1). TIM-3 blockade exacerbates local inflammation and liver damage, increasing expression of TLR4 and chemokine (C-X-C motif) ligands 1 and 2, in a murine model of partial liver warm ischemia (90 minutes) followed by reperfusion, whereas TIM-3 engagement ameliorates neutrophil, T-cell, and macrophage sequestration in IRI livers.[28] The concentrations of Gal-9 in the circulation and hepatic tissues of mice that have

undergone warm ischemia/reperfusion are increased,[29] with hepatic Gal-9 mRNA increasing progressively during reperfusion, peaking at 6 hours and decreasing thereafter. Moreover, the IRI-related damage is profoundly exacerbated in Gal-9-deficient mice compared with wild-type mice, whereas pretreatment with recombinant Gal-9 attenuates liver IRI,[29] profoundly decreasing the expression of TLR4, proinflammatory cytokines (TNF-α, IL-6, IL-1β, and IFN-γ), and apoptosis of hepatocytes. Using mice that selectively overexpress TIM-3 on their T cells (TIM-3Tg) in a clinically relevant liver transplant model, investigators found that higher TIM-3 expression by recipient $CD4^+$ T cells protected liver grafts from IRI.[30] Furthermore, hepatocytes are major Gal-9 producers *in vivo* and within IR-stressed hepatocyte cultures.[30] Taken together, these data point to Gal-9 as an alarmin in liver IRI that provides cytoprotection against tissue damage.[29]

NONALCOHOLIC FATTY LIVER DISEASE

Natural killer T (NKT) cells exhibit features of both innate and adaptive immunity and, although enriched within the normal liver (relative to peripheral compartment), are depleted in nonalcoholic fatty liver disease (NAFLD).[31] Accordingly, up-regulation of hepatic NKT cells has been shown to improve high fat (HF) diet–induced NAFLD and insulin resistance.[32] Gal-9 induces apoptosis of hepatic NKT cells in a dose-dependent fashion and is blocked by α-lactose (competitive inhibitor of Gal-9) or anti-TIM-3 antibody[33]; in mice fed an HF diet, $TIM\text{-}3^{pos}$ NKT cells are more prone to apoptosis. Surprisingly, Gal-9 increases hepatic NKT cells in mice fed an HF diet, selectively enhancing the $CD4^+$ NKT cell population. In the livers of mice fed an HF diet, Gal-9 treatment significantly increased both mRNA and protein expression of IL-15, known to induce proliferation and maintenance of NKT cells. Furthermore, blocking IL-15 or depleting KCs abolished Gal-9-induced hepatic NKT proliferation in these mice.[33] The fact that Gal-9 treatment improved steatosis in mice fed the HF diet underscores the complex roles of the Tim-3/Gal-9 pathway during various phases of the immune response to maintain homeostasis: activation of NKT cells leads to secretion of IFN-γ, which leads to both up-regulation of TIM-3 and production of Gal-9 by KCs that in turn leads to $TIM\text{-}3^+$-dependent and $TIM\text{-}3^+$-independent apoptosis of NKT cells, limiting destructive immunity.[33] Concurrently, Gal-9 also interacts with Tim-3 expressed on KCs to produce IL-15 that induces the proliferation of NKT cells (Fig. 2).

ALCOHOLIC LIVER DISEASE

Recombinant Gal-9 induces proinflammatory mediators such as TNF-α, IL-1β, and IFN-γ from peripheral and liver-derived mononuclear cells of study subjects[15]; and these cytokines are central effectors in alcohol-related liver injury.[34] A recent study found that patients with acute alcoholic hepatitis have higher plasma Gal-9 levels compared to healthy controls or patients with alcoholic cirrhosis.[35] T cells in patients with alcoholic hepatitis expressed higher levels of TIM-3 and Gal-9, correlating with an exaggerated IL-10-mediated antimicrobial T-cell response, which leads to immune exhaustion, and a subsequent failure to support innate immunity, through impaired bacteria-specific IFN-γ responses.[35] These results suggest that a marked immunosuppressive state (compensatory anti-inflammatory response syndrome), rather than the initial proinflammatory cytokine storm, might account for sepsis-related morbidity and mortality in these patients.[35] In a recent study of 575 individuals with at-risk alcohol consumption, we found that *LGALS9* polymorphisms are

associated with development of alcoholic liver disease.[36] The presence of single-nucleotide polymorphisms rs4239242 and rs4794976 is associated with higher transcription and protein expression of Gal-9 following stimulation of monocytes with IFN-γ and ethanol. The seemingly opposite effects of Gal-9 in NAFLD and alcohol-associated liver disease are consistent with the concept that although these two diseases share some aspects of dysregulated innate immunity (including KC activation), distinct immunopathogenic mechanisms are likely operant in NAFLD versus alcoholic liver disease (Fig. 2).

HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, and the majority of cases are linked to chronic infection with viral hepatitis.[37] In HBV-related HCC, Gal-9^{pos} KC and TIM-3^{pos} T cells colocalize, and higher immunohistochemical expression of TIM-3 on CD4$^+$ T cells is associated with shorter survival when compared to the low Tim-3 expressing group, consistent with the concept that Gal-9 impairs liver tumor immunity.[38] Another potential target for Gal-9 in immune suppression is the natural killer (NK) cell population, which comprises important antitumor effectors in the liver. We found that Gal-9 ligation on NK cells down-regulated multiple immune-activating genes, including eight that are involved in the NK cell–mediated cytotoxicity pathway, impaired lymphokine-activated killing, and decreased IFN-γ production independently of Tim-3.[39] Gal-9 is up-regulated at the gene and protein levels in human liver cancer cell lines (HepG2 and SMMC7721) compared to normal hepatocytes (Lo2) and induces apoptosis of peripheral blood mononuclear cells.[40] Furthermore, microRNA 22, which directly inhibits Gal-9 expression, is down-regulated in cancer tissues and in HCC lines.[40] Moreover, up-regulated Gal-9 expression on endothelial cells may also contribute to immune dysfunction.[41] In contrast to the immunosuppressive activities of Gal-9 in promoting tumor escape,[17] Gal-9 also has been associated with antimetastatic potential in HCC, (for example, relative down-regulation of Gal-9 in human HCC was associated with the histopathologic grade of the tumor, lymph node metastasis, vascular invasion, intrahepatic metastasis, and decreased survival),[42] with the likely mechanism that cytoplasmic Gal-9 induces cancer cell aggregation (stabilizing cell–cell adhesion junctions), and inhibits cell invasion, detachment from tumor, and attachment to vascular endothelium (Fig. 3), as shown in breast cancer.[43] Additional antitumor roles for Gal-9 are supported by a recent study in which Gal-9 induced apoptosis of HCC lines *in vitro* in a dose-dependent and time-dependent manner and inhibited the growth of human HCC cells in a xeno-graft athymic murine model.[44] TIM-3-independent apoptosis (without arrest of the cell cycle) occurred through endoplasmic reticulum stress and an intrinsic mitochondrial pathway (caspase-9) involving up-regulation of the oncogene microRNA 1246.[44] Of note, while the apoptotic effect was observed for two HCC lines (HLE and Li-7), Huh-7 cells were resistant, suggesting that not all HCCs will behave the same. These data suggest that Gal-9 holds promise as a potential adjuvant to conventional chemotherapy for some HCCs,[44] particularly because of different mechanisms of action (i.e., sorafenib induces cell cycle arrest). Of interest, Gal-9 also induces apoptosis of cholangiocarcinoma cells *in vitro* and *in vivo* and induces the tumor suppressor microRNA 198 in these cells.[45] The potential duality of Gal-9 in HCC is shown in Fig. 3. It has been proposed that after its initial up-regulation establishes a tolerogenic environment, Gal-9 expression might be lost during tumor progression. Thus, targeting of

Gal-9 may need to be cell-specific to harness the therapeutic potential in the context of HCC.[6]

ACUTE LIVER FAILURE

Acute liver failure (ALF) is comprised of severe liver injury in combination with progressive encephalopathy in a previously healthy patient and is associated with a high risk of progression to multiorgan failure.[46] The mechanisms by which drugs induce liver injury have been incompletely characterized,[47] but the innate immune response has been implicated. KCs play key roles in the development of acetaminophen and non-acetaminophen hepatotoxicity.[48,49] In a recent study, we found that patients (n = 149) with ALF related to either acetaminophen or idiosyncratic non-acetaminophen had higher circulating plasma levels of Gal-9, which was associated with the development of systemic inflammatory response syndrome, and predicted early mortality independently of the Model for End-Stage Liver Disease.[50] We developed competing risk multivariate models that defined the hazard ratios, finding that in ALF patients with identical Model for End-Stage Liver Disease scores, patients with Gal-9 levels ≥690 pg/mL had an almost 3-fold greater risk of dying within 21 days than patients with Gal-9 levels <690. Our results suggest that Gal-9-mediated impairment of immune function might predispose patients to infectious complications because Gal-9 is known to inhibit T-cell[8] and NK-cell[39] function. Development of neutralizing strategies to block Gal-9 effects might provide important insights into pathogenic mechanisms and novel therapeutic targets in the ALF setting.[21]

Summary

Gal-9 has a wide variety of biological functions in innate and adaptive immunity that are instrumental in the maintenance of hepatic homeostasis. Gal-9 may exhibit a “double-edged sword” effect with opposing biological outcomes depending on the localization, cell of origin (e.g., KC, hepatocyte), multiple target cells, and disease process (Table 1). For example, KC-derived Gal-9 favors persistence of viral hepatitis through expansion of Tregs and modulation of Th1 and Th17 immunity through induction of apoptosis. In ischemic liver injury, hepatocyte-derived Gal-9 is both diagnostic and cytoprotective. For Gal-9 to exert therapeutic benefit in NAFLD, it requires driving IL-15 production from KCs, which in turn leads to proliferation of NKT cells. Moreover, emerging work in NAFLD and IRI indicates the possibility that Gal-9 may synergize with TLRs to affect a range of hepatic inflammatory reactions. Ongoing studies are warranted to better elucidate the pathophysiology of hepatic immune-mediated diseases and to develop new therapeutic interventions using glycan-binding proteins.

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Abbreviations

AIH	autoimmune hepatitis

AILD	autoimmune liver disease
ALF	acute liver failure
CD	cluster of differentiation
CRD	carbohydrate recognition domain
Gal-9	galectin-9
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HF	high-fat
IFN-γ	interferon gamma
IL	interleukin
IRI	ischemia/reperfusion injury
KC	Kupffer cell
NAFLD	nonalcoholic fatty liver disease
NK	natural killer
NKT	natural killer T
Th17	T helper 17
TIM-3	T-cell immunoglobulin and mucin domain-containing molecule 3
TLR	Toll-like receptor
TNF-α	tumor necrosis factor alpha
Treg	regulatory T cell

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Author names in bold designate shared co-first authorship.

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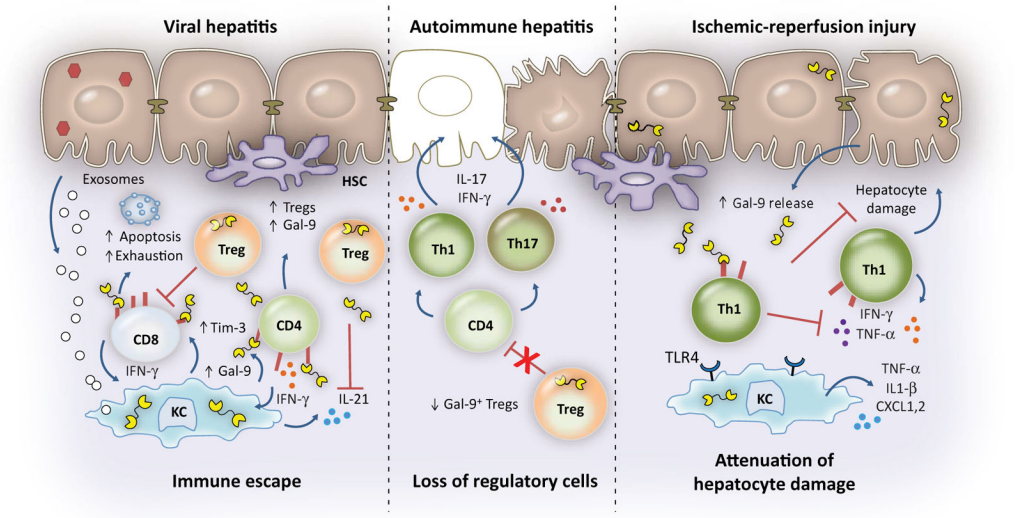

FIG. 1.

Paradigm for Gal-9 in the regulation of immune responses during viral hepatitis infection, AIH, and IRI. Exosomes released from HCV-infected hepatocytes and IFN-γ secreted by the hepatic infiltration of T, NK, and NKT cells induce Gal-9 expression in KCs. Gal-9 binding to Tim-3 induces apoptosis of $CD8^{pos}$ T cells, drives the expansion of Tregs, and suppresses proinflammatory cytokine production, leading to immune escape. Gal-9 expands Tregs (including those that express Gal-9) and leads to contraction of effector $CD4^+$ and $CD8^+$ T cells, including the virus-specific population. Whereas acute, resolving HCV is characterized by strong Th1/Th17 responses and increased levels of IL-21-producing $CD4^+$ T cells and circulating plasma levels of IL-21, persistent infection is associated with increased hepatic and plasma levels of Gal-9; in turn, Gal-9 suppresses IL-21 production, compromising HCV-specific cytotoxic T lymphocyte function by up-regulating exhaustion molecules and becoming more susceptible to apoptosis. Knockdown of Gal-9 in Tregs can rescue IL-21 production by $CD4^+$ T cells. To date, the results for HBV infection (although not as complete) appear similar to those for HCV infection. On the other hand, in AIH, $Gal\text{-}9^{pos}$ Tregs exert control over disease activity, and loss of this population leads to uncontrolled effector proinflammatory IFN-γ and IL-17-producing cells that are the main orchestrators of liver damage in AIH and that correlate with disease severity. For the significant proportion of patients with AILD who fail to respond to conventional treatments, Gal-9/Treg-based immunotherapy may represent a novel approach. In the setting of liver IRI, activation of Th1 cells and of macrophages and neutrophil recruitment occur early. Macrophages elaborate inflammatory cytokines and chemokines and up-regulate TLR4 expression. Gal-9, predominantly released from injured hepatocytes, acts as a cytoprotective alarmin. Binding of Gal-9 to Tim-3 on T cells decreases proinflammatory cytokine production, chemokine responses, and hepatocyte damage; blockade of TIM-3 promotes Th1 responses and exacerbates IRI-triggered liver injury but only in the setting of intact TLR4 signaling.[15,16,18,22,27–30] Abbreviations: CXCL, chemokine (C-X-C motif) ligand; HSC, hepatic stellate cell.

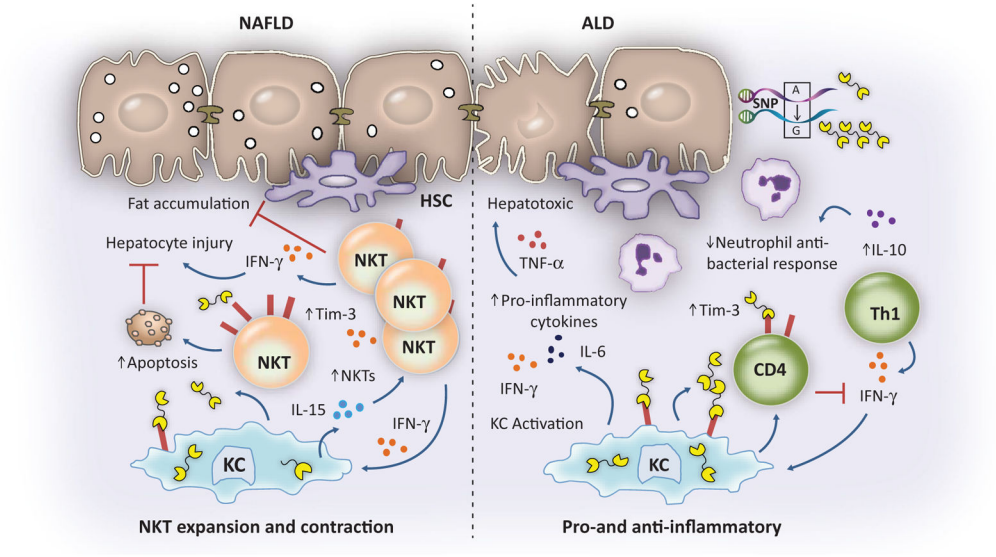

FIG. 2.

Putative roles for Gal-9 in NAFLD and alcohol-associated liver disease. NKT cells are protective in NAFLD. Direct engagement of Gal-9 by Tim-3 expressed on NKT cells induces apoptosis, while Gal-9 increases expression of IL-15 from KCs, which expands the hepatic NKT cell population. Activation of KCs by Gal-9 in the setting of alcoholic liver disease induces several proinflammatory cytokines that have hepatotoxic effects. T cells in patients with alcoholic hepatitis expressed high levels of TIM-3 and Gal-9, correlating with an exaggerated IL-10-mediated antimicrobial T-cell response that leads to immune exhaustion and a subsequent failure to support innate immunity, through impaired bacteria-specific IFN-γ responses. Functional single-nucleotide polymorphisms are associated with development of alcoholic liver disease. Thus, the effects of Gal-9 are disease context–specific.[33,35,36] Abbreviations: ALD, alcoholic liver disease; HSC, hepatic stellate cell; NAFLD, non-alcoholic fatty liver disease; SNP, single-nucleotide polymorphism.

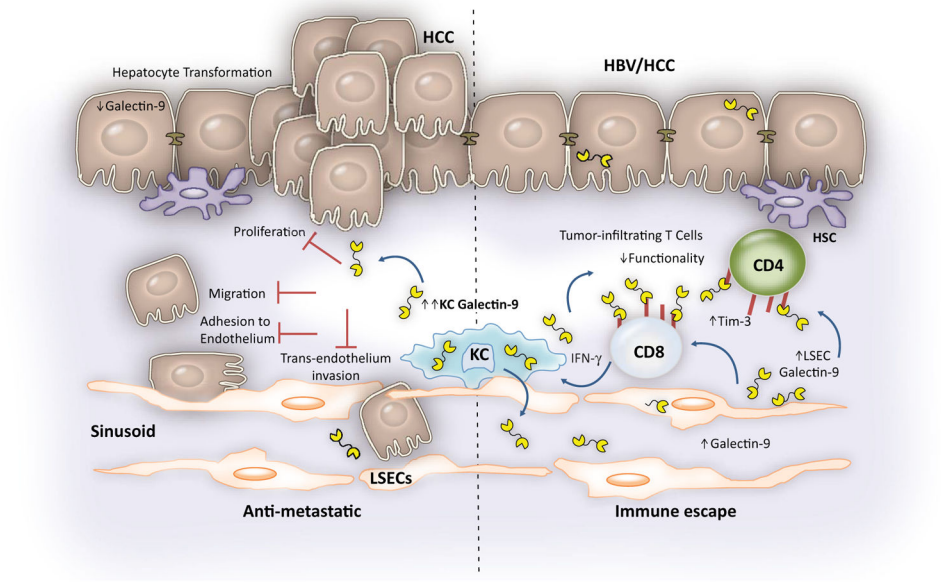

FIG. 3.

The dichotomous roles of Gal-9 in HCC. Gal-9 can display both beneficial and detrimental effects in the context of HCC. KC Gal-9 is highly up-regulated in HBV-associated HCC. In normal liver, Gal-9 is expressed in hepatocytes and down-regulated on transformation. Gal-9 can restore antimetastatic functions by blocking proliferation, migration, adhesion to endothelium, and *trans*-endothelium invasion of carcinoma cells. On the other hand, Gal-9 promotes immune escape by induction of apoptosis and inhibition of effector functions of tumor-infiltrating T cells which express high levels of Tim-3. Increased Gal-9 expression on liver endothelial cells may contribute to immune suppression.[38,39,41,42,44] Abbreviations: HSC, hepatic stellate cell; LSEC, liver sinusoidal endothelial cell.

TABLE 1

Cellular Targets of Gal-9 and Potential Functions in Liver Disease

Cell Population	Function and/or Effect	Role in Liver Disease
T cells	Inhibits proliferation of and cytokine production by $CD4^+$ helper T cells Induces apoptosis of antigen-specific $CD8^+$ T cells Attenuates IFN-γ production by $CD8^+$ T cells	• Involved in suppression of adaptive immunity • Impaired antigen-specific effector function in HCV/HBV leads to chronicity • In AIH, decreased ligand expression renders T cells less prone to immune suppression • In IRI, attenuates local inflammation and liver damage • In ALD, exaggerated IL-10-mediated antimicrobial T-cell response leading to immune exhaustion • In HCC, possible role in the suppression of antitumor immune responses • In ALF, impaired immune function may predispose to infectious complications
Tregs	Expands Tregs Effector molecule	• In HCV/HBV, may lead to immune suppression and chronicity • Failure to exert control over disease activity in AIH; potential role for Gal-9 as therapy • In HCC, possible role in the suppression of antitumor immune responses
NKT cells	Induces apoptosis of NKT cells Expands NKT cells indirectly through up-regulation of IL-15 in macrophages	• In NASH, NKT cells are protective; loss of this population promotes disease progression • Can be protective in NASH, where the expansion of NKTs by IL-15 dominates
KCs	Activation of this population resulting in production of TNF-α, IL-1β, IL-10, and IL-15	• Macrophages and monocytes are predominant cell types producing Gal-9 in many forms of liver disease • Protective in NASH through effect on NKTs • In ALD, may mediate proinflammatory response and liver injury
Hepatocytes	Alarmin produced upon cell injury	• In HCC, suppression promotes cell proliferation and adhesion to ECM, tumor cell–endothelial cell adhesion, and *trans*-endothelial invasion • In IRI, may be cytoprotective

Abbreviations: ALD, alcoholic liver disease; ECM, extracellular matrix; NASH, nonalcoholic steatohepatitis.