Cell Host & Microbe
Perspective

The Liver at the Nexus of Host-Microbial Interactions

Andrew J. Macpherson,[1,*] Mathias Heikenwalder,[2] and Stephanie C. Ganal-Vonarburg[1,*]
[1]Maurice Müller Laboratories (DKF), Universitätsklinik für Viszerale Chirurgie und Medizin Inselspital, Murtenstrasse 35, University of Bern, 3010 Bern, Switzerland
[2]Division of Chronic Inflammation and Cancer, German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany
*Correspondence: andrew.macpherson@insel.ch (A.J.M.), stephanie.ganal@dkf.unibe.ch (S.C.G.-V.)
http://dx.doi.org/10.1016/j.chom.2016.10.016

The liver receives blood from the intestine, from the spleen, and directly from the heart and holds a vital position in vertebrate physiology. It plays a role in intermediary metabolism, bile secretion, maintaining blood sterility, serum homeostasis, xenobiotic detoxification, and immunological activity. This article provides our perspective on the liver as a nexus in establishing and maintaining host microbial mutualism. We discuss the role of the liver not only in sanitizing the blood stream from penetrant live microbes, but also in metabolizing xenobiotics that are synthesized or modified by intestinal microbes, and how microbiota modify the signaling potential of bile acids. The combination of bile acids as hormones and the metabolic control from pervasive effects of other absorbed microbial molecules powerfully shape hepatic metabolism. In addition, intestinal microbial metabolites can be sensed by liver-resident immune cells, which may disturb liver homeostasis, leading to fibrosis and liver cancer.

Prospect

The liver is the heaviest organ and the largest gland in the human body, with an amazing diversity of functions. These can be broadly sub-divided into intermediary metabolism (including a central role in body carbohydrate, lipid, and nitrogen metabolism); secretion of bile; disinfection of the blood facilitated by the largest population of macrophages in the body; sanitation of serum (including synthesis of many serum proteins, degradation of hormones, and detoxification of xenobiotics); and immunological activity as a secondary lymphoid organ. All of these functions have an impact on host-microbial mutualism: the liver not only responds to the microbiota and its metabolites, but also actively shapes intestinal microbial composition and metabolism through enterohepatic biliary circulation.

This article provides our perspective on the central role of the liver in establishing and maintaining host microbial mutualism and its mirroring of some of the consequences of disturbing the host-microbial relationship through diet, intestinal dysfunction, or liver failure. Our initial focus is on blood vascular sanitation from live microbes. We also consider how the body copes with the promiscuous penetration of microbial molecules and how the microbiota modifies the signaling potential of bile acids.

Routes of Penetration of Live Intestinal Microbes or Their Molecular Constituents into the Body

To appreciate the role of the liver in mutualism with the microbiota, one has to distinguish the different routes whereby live microbes from the intestine can penetrate and persist in the body. There are potentially four destinations for any live microbe that penetrates the surface intestinal epithelium: (i) it may be immediately rejected into the intestinal lumen via binding to IgA during intestinal secretion, (ii) it may be destroyed by biocidal activity in intestinal mononuclear cells (macrophages), (iii) it may be sampled by intestinal dendritic cells and transported via the lymph to the local (mesenteric) lymph nodes, or (iv) it may enter the mesenteric bloodstream.

The first two processes of rejection or immediate destruction of the microbe by the host are generally highly efficient provided the host immune system is fully functional and the microbe is non-pathogenic, meaning that it has not developed strategies of evading host immunity, damaging the host itself or occupying a habitat of such intimate apposition with the host epithelium that host compensatory immunity will be continuously challenged.

The third possibility of deliberate lymphatic sampling to induce host immunity requires transit of live intestinal microbes to the mesenteric lymph nodes and uptake by intestinal dendritic or mononuclear cells, which takes place via the afferent lymphatics (Diehl et al., 2013; Macpherson and Uhr, 2004). Only very small numbers of intestinal microbes are sampled, and this occurs without appreciable intestinal damage or inflammation.

The fourth route is live microbial entry into the bloodstream. This requires either direct penetration of the gut vascular endothelial barrier, which is much more likely during intestinal inflammation (Balmer et al., 2014; Spadoni et al., 2015), or a preliminary trajectory of lymphatic spread where organisms such as *Salmonella* are able to establish an early facultative intracellular existence (Dinh et al., 2013).

A clear distinction between the penetration of live microbes and their molecular constituents needs to be made. The number of non-pathogenic live microbes penetrating the body is rather small, even in the context of intestinal inflammation. However, as shown by the sequential uptake of ^{14}C or ^{13}C metabolites from challenge with metabolically labeled bacteria, there is promiscuous exchange of molecules of microbial origin to virtually all tissues of the body (Balmer et al., 2014; Gomez de Agüero et al., 2016). The same conclusion is reached by comparing metabolomic profiles between germ-free and colonized animals (Dumas et al., 2006; Holmes et al., 2011; Marcobal et al., 2015). Both live microbes (Balmer et al., 2014) and their metabolic

constituents and metabolites (Heath and Claus, 2011) require clearance through the liver, but with different potential consequences for the body that will be discussed further in this review.

The Anatomy and Function of the Liver in Relation to the Challenges of Intravascular Microbes and Xenobiotics

Liver Vasculature

To introduce the importance of the liver in terms of mutualism with the intestinal microbiota, we will start with the anatomy of its special blood supply. There are two sources of blood flowing into the liver: (i) arterial blood from the hepatic artery, which branches off the descending aorta via the celiac trunk and (ii) portal venous blood from tributaries of the mesenteric veins that drain the intestinal tract from the stomach to the rectum (Figure 1). Both the hepatic artery and the hepatic portal vein progressively divide into branches bundled together with three other structures: the tributaries of the bile ducts, the lymphatics that drain lymph into the thoracic duct via the hilar lymph nodes, and branches of the vagus nerve. These bundles can be seen as the "portal triads" in liver sections, so named because only the small vessels originating from the hepatic artery, the portal vein, and bile ducts are easy to visualize without special staining (Figure 1A, inset).

The upshot is that the arterial and portal venous blood mix together at a capillary level to flow along the hepatic sinusoids (Figure 1A, inset) draining into the hepatic vein, which is quite distinct and physically separate from the portal vein, and it is the true venous drainage of the liver into the inferior vena cava and the right heart.

The hepatic sinusoids are therefore a filtration system between the arterial and (true) venous blood on one hand, and the portal venous outflow from the intestines and the spleen on the other hand. Expressed in another way, both the gut and the spleen circulations are arranged in series with the liver sinusoids before draining into the venous circulation (Figure 1B).

Hepatic Sinusoids Containing Kupffer Cells—A Site for Vascular Hygiene

The sinusoids are lined with fenestrated "open pore" endothelium, which allows the passage of large molecules, including proteins, into the underlying space of Disse that separates the endothelium of the sinusoids from the hepatocytes.

Within the sinusoids and attached to the endothelium are the Kupffer cells, which are the resident macrophages of the liver, comprising the largest population of macrophages in the body. It is important to appreciate that these cells are highly phagocytic for intravascular bacteria, especially when the bacteria have been opsonized with serum components. The evidence for this comes from papers dating back as far as 100 years and was published in the first volumes of both the *Journal of Immunology* and *Immunology* (Howard and Wardlaw, 1958; Manwaring and Coe, 1916; Manwaring and Fristchen, 1923; Wardlaw and Howard, 1959). The experimental technique used was to perfuse livers ex vivo with physiological solutions of bacterial suspensions containing different concentrations of heat-inactivated or native serum. Uptake of flagellated bacteria was readily measurable from the difference between the bacterial concentrations in the input and output perfusate. Microscopic sections from these experiments showed that the bacterial clearance was due to the uptake of microbes by Kupffer cells. Non-flagellated strains were also generally efficiently taken up, but mainly in the presence of native serum or serum taken from an animal previously immunized with the bacterial strain in question.

This technique also assesses differences in the potential capacity of different tissues to clear bacteria from a perfusate. For example, clearance from the vasculature was shown to be measurably better in the spleen compared with the brain or the lungs. However, a key result was that the most efficient output/input clearance ratio was found by perfusing the liver (Manwaring and Fristchen, 1923). A further finding was that very few bacteria were cleared when perfusing just the intestine, emphasizing that the liver is the critical clearance site for those microbes that have penetrated the intestinal vasculature, a result that aligns with the series arrangement of the mesenteric and liver vascular beds (Figure 1B).

Kupffer cells express an array of scavenger receptors (Armengol et al., 2013) and Fc receptors (FcgammaRII, FcgammaRIII, and in humans FcalphaRI) (Løvdal and Berg, 2001; Monteiro and Van De Winkel, 2003) that enable their phagocytic functions. Complement is also sensed through the presence of complement receptors of the immunoglobulin superfamily (CRIg) on Kupffer cells, which bind fragments C3b and iC3b. There are different subsets of Kupffer cells that can be distinguished according to CD68 and CD11b expression: CRIg is predominantly expressed on CD68+ phagocytic cells rather than CD11b+ cytokine-secreting cells (Ikarashi et al., 2013). Although Kupffer cells dominate hepatic phagocytosis, the endothelial cells lining the sinusoids are also involved in clearance of intravascular particles and express FcgammaRII and (like Kupffer cells) MHCI and MHCII in conjunction with costimulatory molecules for antigen presentation.

Over time experimental techniques have been transformed, so the capture of intravascular bacteria by liver Kupffer cells can now be imaged dynamically (Jenne and Kubes, 2013). The in vivo immunological mechanisms involved have been revealed by combining dynamic imaging with gene-targeted strain combinations and antibody neutralization of protein function. This more detailed appreciation of the mechanisms and different phases of Kupffer cell bacterial uptake from the circulation and further evidence that the liver is usually dominant over the spleen will be described in a later section.

Inflammatory Monocytes and iNKT Cells—Important Immune Mediators in the Liver

Besides resident Kupffer cells, the liver is home to infiltrating monocyte/bone-marrow-derived macrophages. These macrophages enter the liver predominantly in response to acute and chronic liver injury and, compared to rather tolerogenic Kupffer cells, have a more pro-inflammatory profile and can initiate an immune response by activating liver-resident T cells (Ju and Tacke, 2016). A large proportion of lymphocytes residing in the liver are invariant natural killer T (iNKT) cells. These can account for up to 35% of murine and up to 15% of human liver lymphocytes, while they constitute a maximum of 2% of all lymphocytes in other lymphatic organs such as the spleen, lymph nodes, or the intestinal lamina propria, rendering the liver the organ with the highest frequency of iNKT cells (Gao et al., 2009). iNKT cells are a specific subclass of T lymphocytes that carry a semi-invariant T cell receptor using the Vα14-Jα18 α chain in mice and Vα24-Jα18 in men together with a limited number of

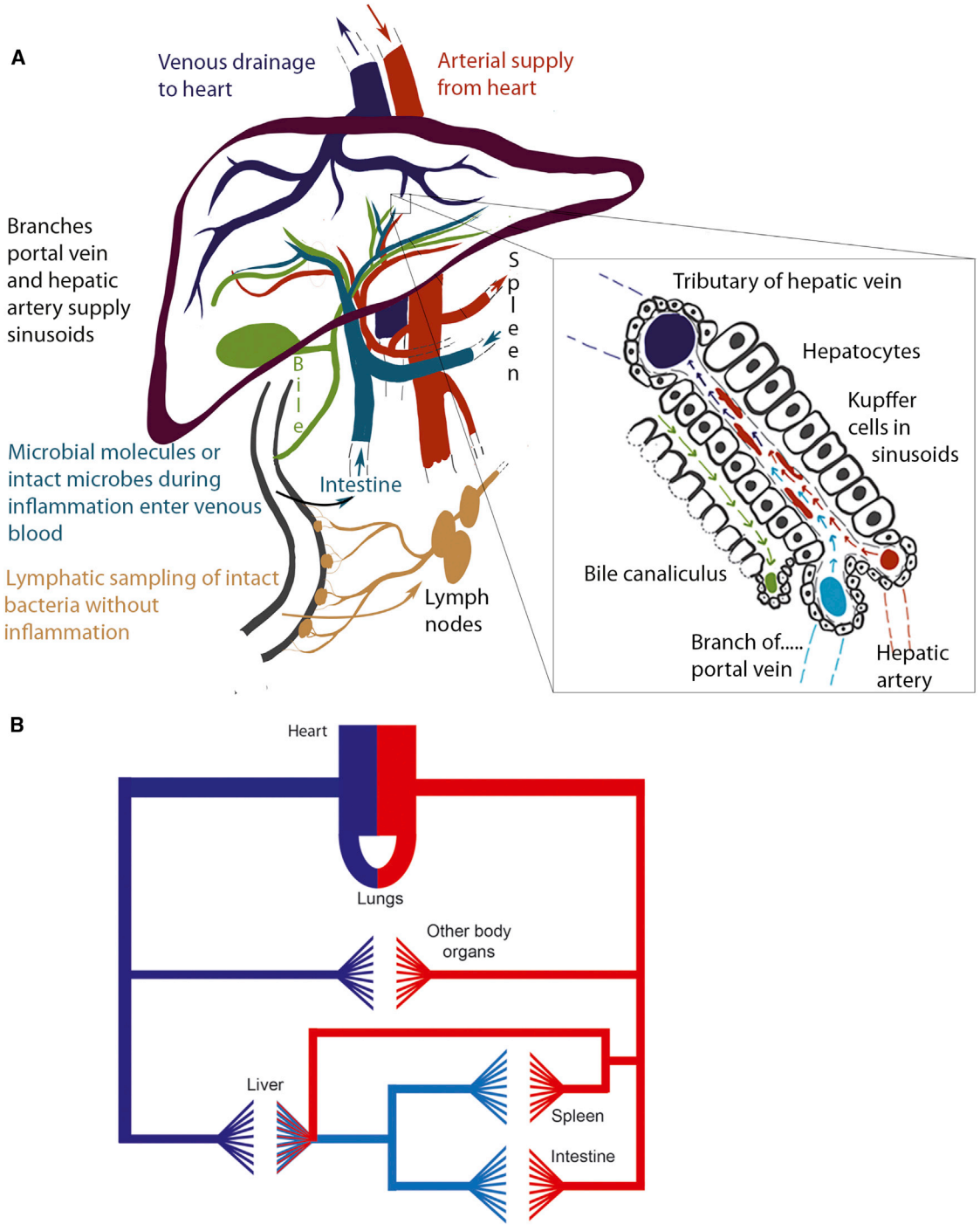

Figure 1. Vasculature and Anatomy of the Liver

(A) Schematic view of liver anatomy and vascularization. The liver receives arterial blood from the hepatic artery, which branches off the descending aorta via the celiac trunk, and portal venous blood from tributaries of the mesenteric veins that drain the intestinal tract. Both the hepatic artery and the hepatic portal vein progressively divide into branches bundled together as the "portal triads" in liver sections: vessels originating from the hepatic artery, the portal vein, and bile ducts. Blood from both sources mix together at a capillary level to flow along the hepatic sinusoids (inset) draining into the hepatic vein and further into the inferior vena cava and the right heart. The sinusoids are lined by fenestrated endothelial cells and contain liver-resident macrophages, the so-called Kupffer cells. Hepatocytes produce primary bile acids that are secreted into the bile canaliculi, eventually reaching the gall bladder.

(B) Schematic view of arterial (red) and venous (blue) blood flow in the body. Note: the liver receives both arterial blood from a branch of the aorta as well as venous blood that has drained from the intestine and the spleens (color appears as a mixture of blue and red), while other organs receive only arterial blood. There are several ways by which intestinal microbes or their metabolites can enter the body from the intestine. Intact bacteria are constantly sampled by dendritic cells in the lamina propria of the intestine and transported to the mesenteric lymph nodes. Some bacteria, and especially bacterial metabolites, can reach the venous blood stream and are transported via the portal vein to the liver. This is much more predominant during damage and/or inflammation of the intestine.

β-chains. iNKT cells are thus limited in their antigen specificity, recognizing glycosphingolipid antigens often present in bacterial cell walls, which are presented by the MHC class I-like molecule CD1d. According to their ability to recognize bacteria-derived components and to be activated immediately, iNKT cells have often been suggested to play an important role in host-microbial mutualism. Blumberg and colleagues reported that germ-free mice harbor more iNKT cells in the intestinal lamina propria and in the airways, while iNKT cell numbers in livers of germ-free mice appear to be unaltered compared to colonized mice (Olszak et al., 2012). In contrast, hepatic iNKT cells seem to be impaired in function in the absence of microbiota, indicating that they do sense signals derived from the commensal microbiota in order to fully mature and play their role in liver homeostasis (Wingender et al., 2012). iNKT cells have been described to play a role in the pathogenesis of various liver diseases, including fibrosis, cancer, viral hepatitis, and even non-alcoholic steatohepatitis and subsequent liver cancer (Dong et al., 2007; Wolf et al., 2014).

Liver Sinusoidal Endothelial Cells and Hepatic Stellate Cells in the Response to Microbial-Derived Antigens

As sentinel cells that encounter intestinal-derived microbial antigens, the stromal cells lining the hepatic sinusoids—namely, liver sinusoidal endothelial cells (LSECs) and hepatic stellate cells (HSCs)—have a key role in regulating the response to microbial antigens. LSECs express high levels of scavenger receptors and compete with liver dendritic cells for the uptake of antigen present in the serum (Schurich et al., 2009). Since they only express low levels of major histocompatibility complex II (MHC II) and co-stimulatory molecules such as CD80 and CD86, LSECs are only weak Th1 cell activators. Rather, they induce both $Foxp3^-$ and $Foxp3^+$ regulatory T cells through secretion of TGF-β, or by inhibiting $CD4^+$ T cells via IL-10 and PD-1 (Carambia et al., 2013). LSECs are also involved in tolerance induction of liver $CD8^+$ T cells (Limmer et al., 2000). Similarly, HSCs provide a number of anti-inflammatory mediators, such as PD-1, IL-10, TGF-β, and B7-H4, that contribute to the regulation of T cell activation (reviewed in Schildberg et al., 2015). These cells are sensing the molecular constituents in blood from the portal vein that is draining the intestine and are at the front line to see a high number of potentially foreign antigens. They are therefore well situated to limit unregulated hepatic T cell activation and potential host organ damage.

Generalized Hepatic Metabolic Functions in Relation to the Position of the Liver

Hepatic anatomy and microanatomy position the liver to filter both the blood from the intestinal tract, spleen, and pancreas via the portal system and the general circulation via its direct arterial supply. This anatomical position is ideal for the liver to clear unwanted microbes from the circulation either directly or in series with the spleen. It is also ideal to fulfill biochemical functions of chemically detoxifying xenobiotics that enter the body through the gut, buffering the blood glucose through hepatic glycogen synthesis and buffering energy stores through biosynthesis or beta oxidation of fatty acids. These three metabolic processes are somewhat connected.

Xenobiotics (chemicals not normally produced by an organism) were initially explored in the early days of organic chemistry, in work that today makes shocking reading (Wöhler and Frerichs, 1848). Animals (and humans) were challenged with organic extracts from plants and products of chemical syntheses such as salicylic acid, benzaldehyde, amygdalin, tannic acid, uric acid, arsenic acid, and phosphoric acid. Modern readers will be horrified by the graphic descriptions of fatal results following deliberate administration of arsenic acid, a non-organic poison that cannot be effectively detoxified: 19th century scientists living in a world of different ethical norms were surprised by the relative lack of toxicity of the other organic compounds and their chemical conversion to apparently innocuous compounds secreted in the urine. The key point that remains relevant today is that these detoxification mechanisms are a combination of host (mainly liver) and microbial metabolism. For example, we now appreciate that Wöhler and Frerichs' observation of chemical conversions of dietary xenobiotics such as salicylic acid and benzaldehyde into hippurate (a benzoic glycine conjugate) involves co-metabolism by intestinal microbes (Claus et al., 2008).

Since the liver is immediately downstream of the intestinal vasculature, it also has the most immediate exposure to bacterially derived molecules, apart from the intestine itself. The portfolio of microbial metabolites is vastly different from true mammalian endobiotics because of the huge diversity of microbial metabolic pathways (Rath and Dorrestein, 2012). Considering the microbiota as part of the host-microbial superorganism somewhat blurs the xenobiotic-endobiotic distinction. Many microbial compounds such as essential amino acids, vitamins, and short-chain fatty acids (SCFAs) are generally beneficial to the host. Nevertheless, aromatics and microbial products of aromatic metabolism are able to exert powerful effects on liver metabolism through members of the nuclear receptor superfamily (farnesoid X receptor [FXR], hepatocyte nuclear factor 4, pregnane X receptor [PXR], and constitutive androstane receptor [CAR]) and the Per/ARNT/Sim family of receptors (aryl hydrocarbon receptor [AhR]; di Masi et al., 2009; Klaassen and Cui, 2015). Colonization of germ-free mice with intestinal microbes increases liver PXR levels and the target gene *Cyp3a11* (Claus et al., 2011). The ligands of PXR are produced by some species within the microbiota (Venkatesh et al., 2014). These receptors have been best investigated as xenosensors capable of regulating the expression of redox, conjugative, and transporter proteins in the liver required to detoxify aromatics and their halogenated derivatives (di Masi et al., 2009). The receptors also have a wider impact on carbohydrate and lipid metabolism, especially in the liver, that connects microbial molecular exchange with central host metabolism: the mechanisms are discussed further in this review.

Immunological and Hematological Mechanisms of Microbial Clearance from the Vasculature

One of the main tasks of the immune system is to clear bloodborne bacteria from the circulation. Failure to do so has catastrophic consequences. The constellation and interactions of microbes, platelets, and complement in the blood potentially triggers disseminated intravascular coagulation, an imbalance in initiation of hemostatic mechanisms over their resolution with intravascular fibrin clots resulting in multiple organ failure. Clinically manifest sepsis is difficult to control, with a high mortality rate (Levy et al., 2015).

In contrast, we are easily able to deal with the low levels of bacteremia that characterize everyday existence, although this

is compromised in patients that have liver disease (Balmer et al., 2014; Kellogg et al., 2000; Zhang et al., 2013). This certainly does depend on the macrophage clearance mechanisms in the liver (Balmer et al., 2014) and the spleen (Aichele et al., 2003). At least for pathogens such as *Listeria monocytogenes* and *Borrelia burgdorferi*, experimental models that compare lack of liver Kupffer cell function with removal of the spleen indicate that the liver has a lion's share of clearing blood-borne infections (Ebe et al., 1999; Lee et al., 2010). Indirect evidence in humans supports the importance of the liver in vascular clearance of microbes. Most patients with serious liver failure as a result of cirrhosis die of sepsis rather than metabolic complications (Leber et al., 2009).

Platelets outnumber blood leukocytes by an order of magnitude. Although their function is best appreciated in terms of their role in hemostasis, they have retained immunological functions seen in multifunctional invertebrate hemocytes such as expression of Toll-like receptors, complement receptors, and adhesion molecules. Platelets also have direct biocidal potential from the secretion of defensins, thrombocidins, and kinocidins (reviewed in Jenne and Kubes, 2015). Dynamic imaging has shown that liver Kupffer cells have large clusters of von Willibrand factor (vWF) decorating their surface. Once Kupffer cells have captured intravascular (pathogenic) bacteria, platelets nucleate on the Kupffer cells: initially there are transient "touch-and-go" interactions between platelet glycoprotein GPIa and vWF, followed later by durable binding between vWF and platelet GPIIb-GPIIIa. The role of each partner in this constellation has been established functionally through bacterial challenge after depletion of Kupffer cells with clodronate liposomes, antibody blockade of vWF, or mouse strain combinations with GP1b deficiency (Wong et al., 2013). Platelet infusion has also been shown to be protective in vivo by an independent group using an *E. coli* model of sepsis (Xiang et al., 2013).

We generally make the assumption that the immune processes essential for pathogen clearance are also functioning more generally to control the smaller numbers of mutualistic microbiota that enter host tissues. This assumption is supported clinically both from opportunistic infections and from primary or acquired immunodeficiency (Taur and Pamer, 2016) or in targeted animal models of immunodeficiency (Shiloh et al., 1999). Given that the perspective of this review is to promote the liver as a central systemic organ of host-microbial mutualism and that the experiments of the Kubes group on platelet nucleation described in the previous paragraph used *Bacillus cereus* and multiple antibiotic-resistant *Staphylococcus aureus* (Wong et al., 2013), we now need to support the argument that this is a general mechanism.

So what is the evidence that the liver is generally clearing the blood vasculature of microbes dominantly over other lymphoid organs such as the spleen? Certainly, the demonstrated superiority of the liver for bacterial clearance in isolated organ infusion experiments cited earlier and clinical observations of septic complications in cirrhotic patients with liver failure are aligned with a general role of this organ in blood disinfection. Splenectomy or splenic dysfunction has potentially serious hematological and immunological consequences, but the susceptibility to infection is limited and centered on encapsulated bacteria such as *Streptococcus pneumoniae*, *Neisseria meningitidis*, or *Haemophilus influenzae* type b (reviewed in Di Sabatino et al., 2011). In these cases, the capsule interferes with complement binding and direct microbial-macrophage binding. The marginal zones of the spleen harbor an IgM B cell memory population, which generate anti-capsule antibodies that opsonize these microbial species for clearance (Kearney et al., 2015). Where it is clear that patients will require surgical splenectomy, the risks of later septic complications can be largely abrogated by immunization with polyvalent pneumococcal polysaccharide vaccine and vaccines against *H. influenzae* and meningococcus.

An independent study by Broadley et al. (2016) examined the clearance of a broad range of bacterial pathogens using in vivo imaging in mice. They compared the trajectory of *Listeria monocytogenes* bacteremia with a range of Gram-postive and Gram-negative microbes. Fast clearance via the Kupffer cells in the liver was confirmed, although a slower mechanism of intravascular platelet binding through GPIb and activated complement C3 later led to capture of the opsonized bacteria with the complement receptor of the immunoglobulin superfamily (Broadley et al., 2016). In this biphasic system, some *Listeria* that escaped the fast clearance mechanism were available to be sampled by CD8α splenic dendritic cells and induced protective T cell immunity (Figure 2), suggesting that the capacity of fast phase clearance sets a threshold for induction of adaptive immunity. One might speculate that this is generally true and can explain the high threshold for the induction of systemic adaptive immunity to benign non-adherent intestinal microbes. Serum antibodies specific to intestinal bacteria are normally only seen in pathogen-free mouse models after experimental systemic priming with the respective bacterium, or where there is background immunodeficiency that would weaken the intestinal protective immune barrier (Konrad et al., 2006; Macpherson et al., 2000; Slack et al., 2009). The concept that the adaptive immune system may be primed to compensate for clearance of otherwise non-pathogenic microbes has also been shown directly in animal models and indirectly in human patients with a range of severity of liver compromise from non-alcoholic steatohepatitis to cirrhosis (Balmer et al., 2014).

Bile Acids as Hormones: Secreted by the Liver and Personalized by an Individual's Intestinal Microbiome

Flowing in the opposite direction to the portal blood supply, but with a ductal system that is bundled with the branches of the portal vein and the hepatic artery, bile is secreted by hepatocytes and cholangiocytes (lining the bile ducts). Within the complex chemical biliary mixture, one finds conjugated primary bile salts that have been synthesized from cholesterol by reorientating the hydroxyl group at the 3 position from a beta to an alpha stereoisomer and by adding hydroxyl groups at the C7 and C12 (cholic acid, hydrophilic) or C7 positions (chenodeoxycholic acid, hydrophobic) and eliminating a proprionyl group from the apical side chain in humans (Chiang, 2009). In rodents, positions 6 and 7 are hydroxylated, giving the relatively hydrophilic muricholic acid. Bile acids are conjugated to glycine or taurine, which decreases toxicity and increases solubility (Figure 3).

The rate-limiting enzyme of this process is microsomal CYP7A1, which catalyzes the C7 hydroxylation, and is inhibited directly or indirectly by insulin, steroid hormones, inflammatory cytokines, growth factors, or bile acids themselves via fibroblast

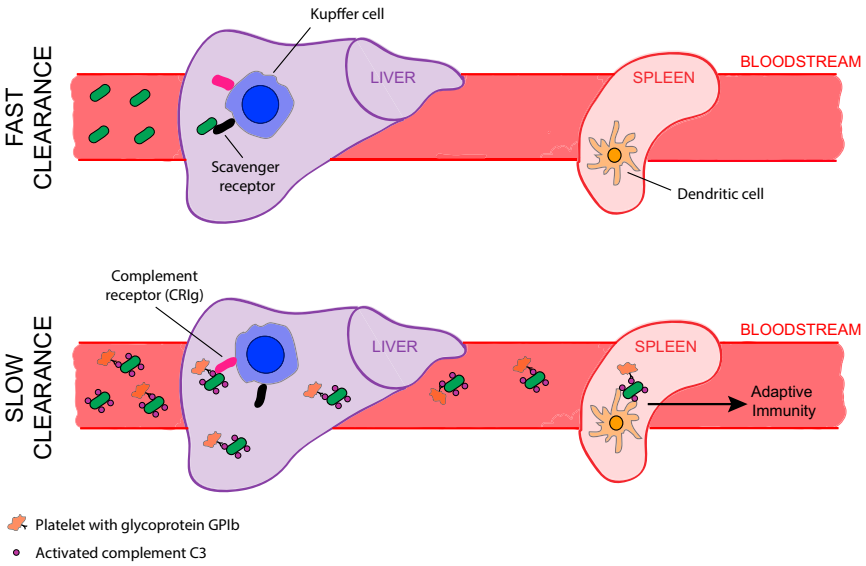

Figure 2. Mechanisms of Bacterial Clearance from Systemic Circulation

Bacteria that enter the bloodstream need to be cleared efficiently to prevent septic shock. There are two main clearing mechanisms that differ in their kinetics, therefore named "fast" and "slow" clearance. Non-opsonized bacteria are efficiently and rapidly cleared by hepatic Kupffer cells via scavenger receptors. Some bacteria are spared from this fast clearance because of opsonization with activated complement C3 and binding to platelets via glycoprotein GPIb. The bacteria-platelet complexes are cleared more slowly via the complement receptor of the immunoglobulin superfamily and are therefore available to dendritic cells in the spleen, which efficiently present antigen to T cells, thereby inducing adaptive immune responses against the invading bacterium.

growth factor (FGF) 15/19 and hepatocyte nuclear factor 4 alpha (Chiang, 2009).

Reabsorption of bile acids in the terminal ileum is extremely efficient (Krag and Phillips, 1974; Schiff et al., 1972), leading to a circulation through the intestine and the liver (Figure 3). This was initially shown by following the trajectory of radiolabeled bile acids over the enterohepatic pathway in experimental animals and man; subsequently, the carrier proteins were characterized (Hofmann et al., 1983; Trauner and Boyer, 2003). A small proportion of bile acids escapes the reabsorption mechanism in the ileum, and once the bile acids have reached the large intestine, different taxa of intestinal bacteria may deconjugate the glycine and taurine side chains or accomplish a variety of redox modifications of which 7 dehydroxylation converts the primary bile acids cholic and chenodeoxycholic acids to deoxycholate and lithocholate, respectively (Doerner et al., 1997; Ridlon et al., 2016; Ridlon et al., 2006). Different members of the genus *Clostridium* have been isolated and shown to convert primary bile acids into secondary bile acids via 7α- dehydroxylation (Ridlon et al., 2006). The genes involved in this process are encoded by a large bile acid inducible (bai) operon (Mallonee et al., 1990; Ridlon et al., 2010; Wells and Hylemon, 2000). These deconjugated secondary bile acids are passively reabsorbed in the colon and return to the liver (Ridlon et al., 2006).

Bile salts have multiple functions including solubilization and absorption of glycerides and cholesterol, promoting cholesterol secretion and bile flow, and acting as anti-bacterial compounds due to their ability to destabilize bacterial membranes. Our perspective of bile salt functions has evolved to include a more hormonal view with pervasive targets affecting host physiology and metabolism. The neoendocrine view involves secretion by the liver, metabolism by the microbiota, and recirculation into the body with a wide range of target organs, including the liver itself (Parséus et al., 2016; Zhou and Hylemon, 2014). This view originated from work showing that bile acids are natural ligands for a wide range of formerly "orphan" nuclear receptors. Before their "adoption" by bile acids, they had been mainly associated with xenobiotic metabolism and excretion, but their importance in vivo for bile acid homeostasis and signaling has been established, including using mouse strains with selective receptor deficiencies (Chiang, 2009; di Masi et al., 2009; Sinal et al., 2000). These nuclear receptors (that form a family with steroid and thyroid hormone receptors and have a promiscuous ligand portfolio) include the farnesoid X receptor, pregnane X receptor, vitamin D receptor, and G protein-coupled receptors (TGR5, sphingosine-1-phosphate receptor 2, muscarinic receptor 2) (di Masi et al., 2009).

Different bile acids vary considerably in their ability to activate each of these receptors (Zhou and Hylemon, 2014), so the personalized details of bile acid metabolism, which depend on the composition of the microbiota and the diet in any individual (Philipp, 2011), likely make a big difference to the extent of receptor signaling. For example, only a relatively narrow spectrum of bacterial species encode enzymatic capability for 7-dehydroxylation or 3-epimerization (Devlin and Fischbach, 2015; Ridlon et al., 2006, 2010). The individualized specifics of the bile salt portfolio thus has far-reaching consequences of multiple targets that influence intestinal physiology and central metabolism, including lipid metabolism (Sinal et al., 2000), glucose homeostasis (Kuipers et al., 2014), fatty acid biosynthesis or beta oxidation, brown fat formation, and negative feedback effects on bile salt secretion itself (Duboc et al., 2014). There are also direct effects on the immune system; for example, TGR5 is expressed by macrophages including the Kupffer cells in the liver (Keitel et al., 2008). Ligation of TGR5 by hydrophobic bile acids, such as lithocholic or deoxycholic acids, downregulates the TNF inflammatory response to LPS challenge (Kawamata et al., 2003).

Microbial Metabolism and Proinflammatory Effects Alter Lipid and Carbohydrate Metabolism in the Liver

Effects of the Microbiota on Increasing the Energy Harvest

Comparisons of germ-free and colonized animals show that the presence of an intestinal microbiota increases the lipid content of hepatocytes and decreases the content of glycogen (Bäckhed et al., 2004; Chuang et al., 2012). In seminal studies, Bäckhed and colleagues showed that colonization increases overall

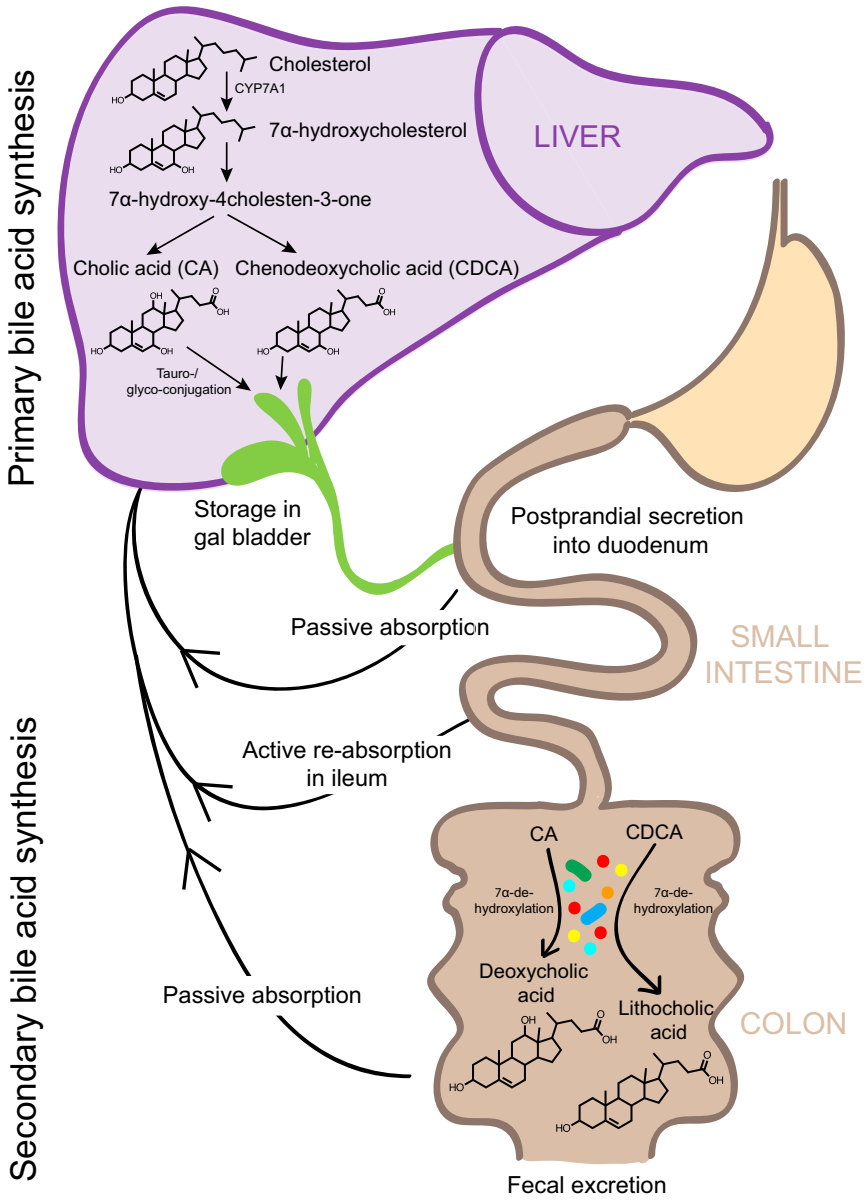

Figure 3. Bile Acid Synthesis, Secretion, and Recirculation in Humans

Primary bile acid synthesis takes place within hepatocytes through a multistep process from cholesterol. The first step in this process is catabolized by the enzyme CYP7A1, converting cholesterol into 7α-hydroxycholesterol, and is the rate-limiting step in the synthesis of primary bile acids. In humans, several additional steps are necessary to generate either cholic acid (CA) or chenodeoxycholic acid (CDCA). Before secretion into the bile duct, CA and CDCA are conjugated to one of the two amino acids, glycine or taurine, to increase their solubility in the acidic milieu of the duodenum. Conjugated bile acids are concentrated and stored in the gall bladder before being released into the duodenum postprandially, where they are important factors in the digestion of dietary fat. Most primary bile acids are actively re-absorbed in the terminal ileum and returned to the liver. Few bile acids escape this process and are deconjugated and converted into deoxycholic acid and lithocholic acid by intestinal microbiota in the large intestine. These secondary bile acids are either passively re-absorbed and transported back to the liver or excreted via feces.

body fat, despite reduced caloric intake with loss of insulin sensitivity and no decrease of the overall metabolic rate (Bäckhed et al., 2004). A mechanism of this effect was found to be an acceleration of lipogenesis in the liver. The intestinal microbiota is metabolically capable of breaking down polysaccharide glycosidic bonds that are resistant to host digestion. This results in an increased release of simple sugars from dietary sources, which are taken up by the host, and hence an increased energy harvest from the diet. A second effect that increases the delivery of digested saccharides to the liver in animals hosting a microbiota is the expansion of the intestinal vascular capillary bed as a result of colonization (Stappenbeck et al., 2002). The increased absorption of these sugars in colonized mice promoted lipogenesis via signaling through the sterol response element binding protein 1 (SREBP1) and upregulating key enzymes of the fatty acid synthesis pathway.

Effects of the Microbiota on Signaling

However, while gut bacterial colonization increased the lipid content of the liver, it also caused overall fat increase in adipose tissue throughout the body. The mechanism for this was not due to de novo differentiation and increase of adipocytes, but rather was due to loading of adipocytes with lipid through increased activity of lipases, the enzymes that release fatty acids from lipoprotein complexes in the circulation (Bäckhed et al., 2004). Lipoprotein lipase is downregulated by fasting-induced adipose factor (FIAF, otherwise called angiopoietin-4) expressed by the intestine, liver, and white and brown fat. Microbial colonization decreases FIAF expression, resulting in increased fatty acid synthesis in the liver and increased fat dissemination throughout the body.

Other effects of the microbiota affecting the control of lipid and carbohydrate metabolism in the liver include inflammation, signaling from bile acids, and direct effects of other microbial metabolites (Parséus et al., 2016). For example, microbiota composition can change and become proinflammatory in the context of defective inflammasome function, resulting in increased Toll-like receptor 4 (TLR4) and TLR9 ligands in the portal circulation driving inflammatory cytokine expression in the liver (Henao-Mejia et al., 2012). These proinflammatory effects activate gluconeogenesis (Figure 4) and increase insulin resistance through transcriptional inhibition of *Cyp7a3*, the rate-limiting enzyme of the neutral bile salt pathway. This promotes accumulation of the pathway precursors farnesyl- and geranylgeranylpyrophospate, which sets up isoprenylation reactions

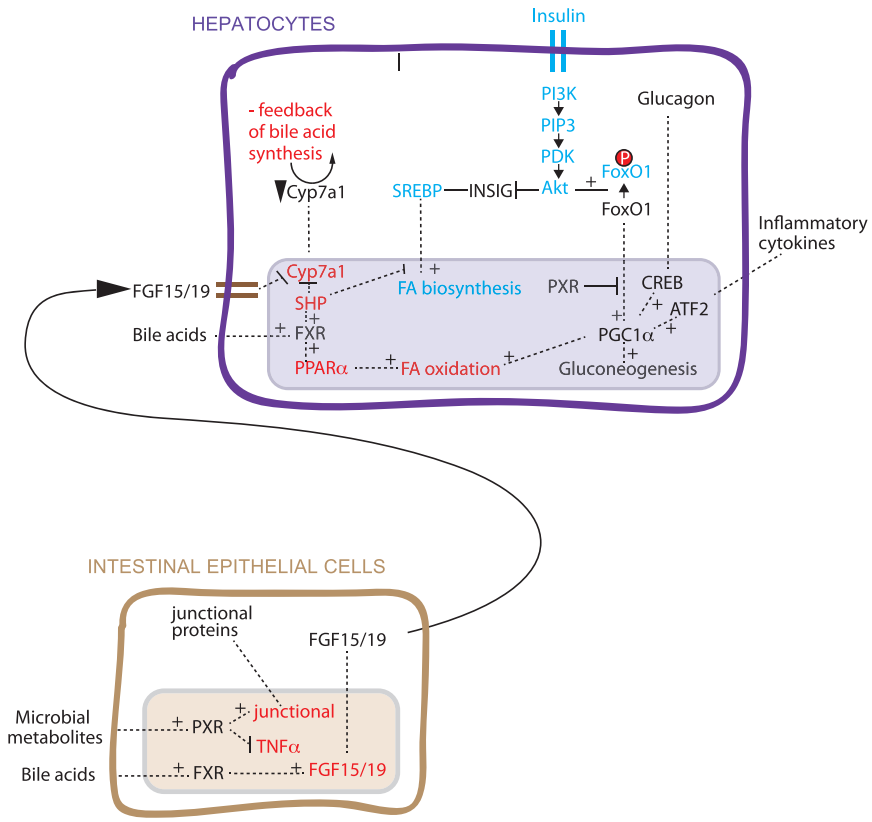

Figure 4. Metabolic Alterations Driven by Microbial Metabolites and Bile Acids

Insulin signaling is shown in blue, and bile acid and microbial metabolite signaling in red. For simplicity, signaling partner proteins are not shown and nuclear transcriptional controls are summarized according to the pathway involved. Akt, serine/threonine protein kinase B; ATF2, activating transcription factor 2; CREB, cAMP response element binding protein; *Cyp7a1*, cytochrome P450 7a1 (cholesterol 7 α hydroxylase); FGF15/19, fibroblast growth factor 15 (mouse) 19 (human); FoxO, forkhead box protein O1; FXR, farnesyl X receptor; INSIG, insulin induced gene 1; PDK, phosphoinositide-dependent kinase; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1- α; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PXR, pregnane X receptor; SHP, small heterodimer partner; SREBP, sterol regulatory element-binding protein; TNF-α, tumor necrosis factor α.

that culminate in insulin receptor inhibition. Conversely, bile salt signaling via FXR has been shown in FXR-deficient mice and through FXR agonist experiments to decrease hepatic fatty acid biosynthesis and increase beta oxidation (Figure 4) (Jiao et al., 2015).

Epigenetic Activity of Dietary Bacterially Metabolized Compounds

Some diet and microbiota-dependent alterations in liver physiology likely occur via epigenetic non-heritable changes including DNA methylation, covalent histone modifications, and regulation of gene expression by non-coding RNAs. Direct molecular products of digestion and indirect products after microbial metabolism can have epigenetic potential, either by providing substrates for covalent modifications such as methylation and acetylation or by generating compounds that directly influence the activity of enzymes involved in epigenetic modifications. The intestine delivers high concentrations of these compounds to the liver via the portal blood. We will take a few examples from this rapidly developing area to indicate its likely breadth and the potential interplay of intestinal microbes.

SCFAs, such as butyrate, propionate, and acetate, generated by some intestinal microbes through the fermentation of fiber, are found in both the colonic epithelium and the portal blood (Cummings and Macfarlane, 1991; Cummings et al., 1987). SCFAs act as histone deacetylase (HDAC) inhibitors and are a well-described system to generate epigenetic marks (Maolanon et al., 2016). Dietary glucosinolates present in cruciferous vegetables can be converted to isothiocyanates (ITCs), which also have epigenetic potential for HDAC inhibition. Conversion by gut bacterial thioglucosidases is especially important after ingestion of cooked vegetables, as plant-derived myrosinases are inactivated by the cooking. A number of intestinal microbial species including *Escherichia coli*, *Bacteroides thetaiotaomicron*, *Enterococcus faecalis*, *Enterococcus faecium*, *Peptostreptococcus sp.*, and *Bifidobacterium sp.* have the capacity to convert glucosinoates into ITCs (Brabban and Edwards, 1994; Elfoul et al., 2001) with absorption into the liver and systemic tissues (Holst and Williamson, 2004).

Polyphenols are phytochemicals that comprise several classes according to their chemical structures. They include phenolic acids, anthocyanins, flavonoids, stilbenes, lignans, and curcuminoids. These compounds are also metabolized by bacteria in the large intestine and enter enterohepatic circulation. Many of the dietary polyphenolic compounds have been associated with epigenetic alterations, and it is likely that the microbially transformed polyphenols also have such potential (Hullar and Fu, 2014). Finally, a link between bile acid nuclear receptor signaling and epigenetic marks has been made through the observation that lysine-specific histone demethylase 1 is induced by FXR activity (Kim et al., 2015).

Signaling from the Microbiota Potentially Causing Liver Fibrosis and Cancer

The impact of the microbiota on fibrosis and hepatic malignant potential has been a long-standing issue. As we have described, bacterial products or PAMPs can drive liver inflammation and liver metabolism. Here, we will specifically consider the effects of signaling from TLR4, which detects lipopolysaccharides (LPS) from Gram-negative bacteria and is expressed by almost all cell types of the liver including hepatocytes (Liu et al., 2002), Kupffer cells (Su et al., 2000), stellate cells (Paik et al., 2003), sinusoidal endothelial cells (Uhrig et al., 2005), and biliary epithelial cells (Harada et al., 2003). In alcohol-induced liver injury, activation of Kupffer cells is closely linked to TLR4 expression (Uesugi

et al., 2001), and in a methionine-choline-deficient dietary model of non-alcoholic fatty liver disease TLR4 absence limits liver inflammation and hepatic lipid accumulation (Rivera et al., 2007). In addition, alcohol consumption is linked to lower intestinal anti-microbial peptide (REG-3 lectins) expression, which leads to translocation of commensal bacteria to the mesenteric lymph nodes and the liver, enhancing the progression of alcohol-induced liver injury (Wang et al., 2016). Hepatic fibrosis and liver cirrhosis have also been associated with TLR4; TLR4-deficient mice showed less fibrosis in chemically or bile duct ligation-induced fibrosis models (Seki et al., 2007).

Persistent inflammation is known to increase the chances of hepatic malignancy. TLR4-dependent participation in hepatocellular carcinoma (HCC) has also been found in mice, contributing to the evidence that HCC is promoted by the intestinal microbiota (Dapito et al., 2012). The liver tumors in chronically injured livers in this study depended on the gut microbiota and TLR4 activation in resident liver cells—both hepatocytes and HSCs. In contrast, Yu and colleagues found that Kupffer cells were the main hepatic targets of TLR4 agonists that induced TNF-α and IL-6 (Yu et al., 2010), both of which can drive compensatory proliferation and also tumor formation (Naugler et al., 2007). In humans, a single nucleotide variation in the *TLR4* gene is associated with protection against fibrosis and liver cancer progression in the context of hepatitis C virus infection (Agúndez et al., 2012; Guo et al., 2009).

Retrospect

In this article, we have related the anatomic intimacy of the liver and intestine with its equally intimate role in host microbial mutualism. Not only does the liver function as a vascular firewall for sanitizing those microbes that reach the portal vein or the systemic vasculature, but it must metabolize or detoxify the tsunami of molecules emanating from direct dietary absorption or after secondary microbial conversion. Bile acid synthesis and metabolism have long been known to be shared between the liver and the intestinal microbiota, although bile salts are now appreciated to have significant neoendocrine signaling roles. With the liver at the very center of host-microbial interactions, including mutualism, it represents an area of study where the concepts of toxicology, microbiology, immunology, physiology, and metabolism meet.

ACKNOWLEDGMENTS

The work of A.J.M. is funded by the Swiss National Science Foundation (SNSF 310030B_160262, SNSF Sinergia CRSII3_154414) and the Swiss SystemsX program (GutX). M.H. is funded by a grant of the European Research Council (Hepatometabopath) and grants of the German Research Foundation (SFBTR36 and SFBTR179). S.C.G.-V. is a holder of a long-term fellowship from the European Molecular Biology Organization (ALTF 841-2013).

REFERENCES

Agúndez, J.A., García-Martín, E., Devesa, M.J., Carballo, M., Martínez, C., Lee-Brunner, A., Fernández, C., Díaz-Rubio, M., and Ladero, J.M. (2012). Polymorphism of the TLR4 gene reduces the risk of hepatitis C virus-induced hepatocellular carcinoma. Oncology *82*, 35–40.

Aichele, P., Zinke, J., Grode, L., Schwendener, R.A., Kaufmann, S.H., and Seiler, P. (2003). Macrophages of the splenic marginal zone are essential for trapping of blood-borne particulate antigen but dispensable for induction of specific T cell responses. J. Immunol. *171*, 1148–1155.

Armengol, C., Bartolí, R., Sanjurjo, L., Serra, I., Amézaga, N., Sala, M., and Sarrias, M.R. (2013). Role of scavenger receptors in the pathophysiology of chronic liver diseases. Crit. Rev. Immunol. *33*, 57–96.

Bäckhed, F., Ding, H., Wang, T., Hooper, L.V., Koh, G.Y., Nagy, A., Semenkovich, C.F., and Gordon, J.I. (2004). The gut microbiota as an environmental factor that regulates fat storage. Proc. Natl. Acad. Sci. USA *101*, 15718–15723.

Balmer, M.L., Slack, E., de Gottardi, A., Lawson, M.A., Hapfelmeier, S., Miele, L., Grieco, A., Van Vlierberghe, H., Fahrner, R., Patuto, N., et al. (2014). The liver may act as a firewall mediating mutualism between the host and its gut commensal microbiota. Sci. Transl. Med. *6*, 237ra66.

Brabban, A.D., and Edwards, C. (1994). Isolation of glucosinolate degrading microorganisms and their potential for reducing the glucosinolate content of rapemeal. FEMS Microbiol. Lett. *119*, 83–88.

Broadley, S.P., Plaumann, A., Coletti, R., Lehmann, C., Wanisch, A., Seidlmeier, A., Esser, K., Luo, S., Rämer, P.C., Massberg, S., et al. (2016). Dual-Track Clearance of Circulating Bacteria Balances Rapid Restoration of Blood Sterility with Induction of Adaptive Immunity. Cell Host Microbe *20*, 36–48.

Carambia, A., Frenzel, C., Bruns, O.T., Schwinge, D., Reimer, R., Hohenberg, H., Huber, S., Tiegs, G., Schramm, C., Lohse, A.W., and Herkel, J. (2013). Inhibition of inflammatory CD4 T cell activity by murine liver sinusoidal endothelial cells. J. Hepatol. *58*, 112–118.

Chiang, J.Y. (2009). Bile acids: regulation of synthesis. J. Lipid Res. *50*, 1955–1966.

Chuang, H.L., Huang, Y.T., Chiu, C.C., Liao, C.D., Hsu, F.L., Huang, C.C., and Hou, C.C. (2012). Metabolomics characterization of energy metabolism reveals glycogen accumulation in gut-microbiota-lacking mice. J. Nutr. Biochem. *23*, 752–758.

Claus, S.P., Tsang, T.M., Wang, Y., Cloarec, O., Skordi, E., Martin, F.P., Rezzi, S., Ross, A., Kochhar, S., Holmes, E., and Nicholson, J.K. (2008). Systemic multicompartmental effects of the gut microbiome on mouse metabolic phenotypes. Mol. Syst. Biol. *4*, 219.

Claus, S.P., Ellero, S.L., Berger, B., Krause, L., Bruttin, A., Molina, J., Paris, A., Want, E.J., de Waziers, I., Cloarec, O., et al. (2011). Colonization-induced host-gut microbial metabolic interaction. MBio *2*, e00271–e10.

Cummings, J.H., and Macfarlane, G.T. (1991). The control and consequences of bacterial fermentation in the human colon. J. Appl. Bacteriol. *70*, 443–459.

Cummings, J.H., Pomare, E.W., Branch, W.J., Naylor, C.P., and Macfarlane, G.T. (1987). Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut *28*, 1221–1227.

Dapito, D.H., Mencin, A., Gwak, G.Y., Pradere, J.P., Jang, M.K., Mederacke, I., Caviglia, J.M., Khiabanian, H., Adeyemi, A., Bataller, R., et al. (2012). Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. Cancer Cell *21*, 504–516.

Devlin, A.S., and Fischbach, M.A. (2015). A biosynthetic pathway for a prominent class of microbiota-derived bile acids. Nat. Chem. Biol. *11*, 685–690.

di Masi, A., De Marinis, E., Ascenzi, P., and Marino, M. (2009). Nuclear receptors CAR and PXR: Molecular, functional, and biomedical aspects. Mol. Aspects Med. *30*, 297–343.

Di Sabatino, A., Carsetti, R., and Corazza, G.R. (2011). Post-splenectomy and hyposplenic states. Lancet *378*, 86–97.

Diehl, G.E., Longman, R.S., Zhang, J.X., Breart, B., Galan, C., Cuesta, A., Schwab, S.R., and Littman, D.R. (2013). Microbiota restricts trafficking of bacteria to mesenteric lymph nodes by CX(3)CR1(hi) cells. Nature *494*, 116–120.

Dinh, T., Ladabaum, U., Alperin, P., Caldwell, C., Smith, R., and Levin, T.R. (2013). Health benefits and cost-effectiveness of a hybrid screening strategy for colorectal cancer. Clin. Gastroenterol. Hepatol. *11*, 1158–1166.

Doerner, K.C., Takamine, F., LaVoie, C.P., Mallonee, D.H., and Hylemon, P.B. (1997). Assessment of fecal bacteria with bile acid 7 alpha-dehydroxylating activity for the presence of bai-like genes. Appl. Environ. Microbiol. *63*, 1185–1188.

Dong, Z., Wei, H., Sun, R., and Tian, Z. (2007). The roles of innate immune cells in liver injury and regeneration. Cell. Mol. Immunol. *4*, 241–252.

Duboc, H., Taché, Y., and Hofmann, A.F. (2014). The bile acid TGR5 membrane receptor: from basic research to clinical application. Dig. Liver Dis. *46*, 302–312.

Dumas, M.E., Barton, R.H., Toye, A., Cloarec, O., Blancher, C., Rothwell, A., Fearnside, J., Tatoud, R., Blanc, V., Lindon, J.C., et al. (2006). Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. Proc. Natl. Acad. Sci. USA *103*, 12511–12516.

Ebe, Y., Hasegawa, G., Takatsuka, H., Umezu, H., Mitsuyama, M., Arakawa, M., Mukaida, N., and Naito, M. (1999). The role of Kupffer cells and regulation of neutrophil migration into the liver by macrophage inflammatory protein-2 in primary listeriosis in mice. Pathol. Int. *49*, 519–532.

Elfoul, L., Rabot, S., Khelifa, N., Quinsac, A., Duguay, A., and Rimbault, A. (2001). Formation of allyl isothiocyanate from sinigrin in the digestive tract of rats monoassociated with a human colonic strain of Bacteroides thetaiotaomicron. FEMS Microbiol. Lett. *197*, 99–103.

Gao, B., Radaeva, S., and Park, O. (2009). Liver natural killer and natural killer T cells: immunobiology and emerging roles in liver diseases. J. Leukoc. Biol. *86*, 513–528.

Gomez de Agüero, M., Ganal-Vonarburg, S.C., Fuhrer, T., Rupp, S., Uchimura, Y., Li, H., Steinert, A., Heikenwalder, M., Hapfelmeier, S., Sauer, U., et al. (2016). The maternal microbiota drives early postnatal innate immune development. Science *351*, 1296–1302.

Guo, J., Loke, J., Zheng, F., Hong, F., Yea, S., Fukata, M., Tarocchi, M., Abar, O.T., Huang, H., Sninsky, J.J., and Friedman, S.L. (2009). Functional linkage of cirrhosis-predictive single nucleotide polymorphisms of Toll-like receptor 4 to hepatic stellate cell responses. Hepatology *49*, 960–968.

Harada, K., Ohira, S., Isse, K., Ozaki, S., Zen, Y., Sato, Y., and Nakanuma, Y. (2003). Lipopolysaccharide activates nuclear factor-kappaB through toll-like receptors and related molecules in cultured biliary epithelial cells. Lab. Invest. *83*, 1657–1667.

Heath, P., and Claus, S.P. (2011). Assessing hepatic metabolic changes during progressive colonization of germ-free mouse by 1H NMR spectroscopy. J. Vis. Exp. (58), 3642.

Henao-Mejia, J., Elinav, E., Jin, C., Hao, L., Mehal, W.Z., Strowig, T., Thaiss, C.A., Kau, A.L., Eisenbarth, S.C., Jurczak, M.J., et al. (2012). Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature *482*, 179–185.

Hofmann, A.F., Molino, G., Milanese, M., and Belforte, G. (1983). Description and simulation of a physiological pharmacokinetic model for the metabolism and enterohepatic circulation of bile acids in man. Cholic acid in healthy man. J. Clin. Invest. *71*, 1003–1022.

Holmes, E., Li, J.V., Athanasiou, T., Ashrafian, H., and Nicholson, J.K. (2011). Understanding the role of gut microbiome-host metabolic signal disruption in health and disease. Trends Microbiol. *19*, 349–359.

Holst, B., and Williamson, G. (2004). A critical review of the bioavailability of glucosinolates and related compounds. Nat. Prod. Rep. *21*, 425–447.

Howard, J.G., and Wardlaw, A.C. (1958). The opsonic effect of normal serum on the uptake of bacteria by the reticulo-endothelial system; perfusion studies with isolated rat liver. Immunology *1*, 338–352.

Hullar, M.A., and Fu, B.C. (2014). Diet, the gut microbiome, and epigenetics. Cancer J. *20*, 170–175.

Ikarashi, M., Nakashima, H., Kinoshita, M., Sato, A., Nakashima, M., Miyazaki, H., Nishiyama, K., Yamamoto, J., and Seki, S. (2013). Distinct development and functions of resident and recruited liver Kupffer cells/macrophages. J. Leukoc. Biol. *94*, 1325–1336.

Jenne, C.N., and Kubes, P. (2013). Immune surveillance by the liver. Nat. Immunol. *14*, 996–1006.

Jenne, C.N., and Kubes, P. (2015). Platelets in inflammation and infection. Platelets *26*, 286–292.

Jiao, Y., Lu, Y., and Li, X.Y. (2015). Farnesoid X receptor: a master regulator of hepatic triglyceride and glucose homeostasis. Acta Pharmacol. Sin. *36*, 44–50.

Ju, C., and Tacke, F. (2016). Hepatic macrophages in homeostasis and liver diseases: from pathogenesis to novel therapeutic strategies. Cell. Mol. Immunol. *13*, 316–327.

Kawamata, Y., Fujii, R., Hosoya, M., Harada, M., Yoshida, H., Miwa, M., Fukusumi, S., Habata, Y., Itoh, T., Shintani, Y., et al. (2003). A G protein-coupled receptor responsive to bile acids. J. Biol. Chem. *278*, 9435–9440.

Kearney, J.F., Patel, P., Stefanov, E.K., and King, R.G. (2015). Natural antibody repertoires: development and functional role in inhibiting allergic airway disease. Annu. Rev. Immunol. *33*, 475–504.

Keitel, V., Donner, M., Winandy, S., Kubitz, R., and Häussinger, D. (2008). Expression and function of the bile acid receptor TGR5 in Kupffer cells. Biochem. Biophys. Res. Commun. *372*, 78–84.

Kellogg, J.A., Manzella, J.P., and Bankert, D.A. (2000). Frequency of low-level bacteremia in children from birth to fifteen years of age. J. Clin. Microbiol. *38*, 2181–2185.

Kim, Y.C., Fang, S., Byun, S., Seok, S., Kemper, B., and Kemper, J.K. (2015). Farnesoid X receptor-induced lysine-specific histone demethylase reduces hepatic bile acid levels and protects the liver against bile acid toxicity. Hepatology *62*, 220–231.

Klaassen, C.D., and Cui, J.Y. (2015). Review: Mechanisms of How the Intestinal Microbiota Alters the Effects of Drugs and Bile Acids. Drug Metab. Dispos. *43*, 1505–1521.

Konrad, A., Cong, Y., Duck, W., Borlaza, R., and Elson, C.O. (2006). Tight mucosal compartmentation of the murine immune response to antigens of the enteric microbiota. Gastroenterology *130*, 2050–2059.

Krag, E., and Phillips, S.F. (1974). Active and passive bile acid absorption in man. Perfusion studies of the ileum and jejunum. J. Clin. Invest. *53*, 1686–1694.

Kuipers, F., Bloks, V.W., and Groen, A.K. (2014). Beyond intestinal soap–bile acids in metabolic control. Nat. Rev. Endocrinol. *10*, 488–498.

Leber, B., Mayrhauser, U., Rybczynski, M., and Stadlbauer, V. (2009). Innate immune dysfunction in acute and chronic liver disease. Wien. Klin. Wochenschr. *121*, 732–744.

Lee, W.Y., Moriarty, T.J., Wong, C.H., Zhou, H., Strieter, R.M., van Rooijen, N., Chaconas, G., and Kubes, P. (2010). An intravascular immune response to Borrelia burgdorferi involves Kupffer cells and iNKT cells. Nat. Immunol. *11*, 295–302.

Levy, M.M., Rhodes, A., Phillips, G.S., Townsend, S.R., Schorr, C.A., Beale, R., Osborn, T., Lemeshow, S., Chiche, J.D., Artigas, A., and Dellinger, R.P. (2015). Surviving Sepsis Campaign: association between performance metrics and outcomes in a 7.5-year study. Crit. Care Med. *43*, 3–12.

Limmer, A., Ohl, J., Kurts, C., Ljunggren, H.G., Reiss, Y., Groettrup, M., Momburg, F., Arnold, B., and Knolle, P.A. (2000). Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. Nat. Med. *6*, 1348–1354.

Liu, S., Gallo, D.J., Green, A.M., Williams, D.L., Gong, X., Shapiro, R.A., Gambotto, A.A., Humphris, E.L., Vodovotz, Y., and Billiar, T.R. (2002). Role of toll-like receptors in changes in gene expression and NF-kappa B activation in mouse hepatocytes stimulated with lipopolysaccharide. Infect. Immun. *70*, 3433–3442.

Løvdal, T., and Berg, T. (2001). Transcription of Fc(gamma) receptors in different rat liver cells. Cell Biol. Int. *25*, 821–824.

Macpherson, A.J., and Uhr, T. (2004). Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. Science *303*, 1662–1665.

Macpherson, A.J., Gatto, D., Sainsbury, E., Harriman, G.R., Hengartner, H., and Zinkernagel, R.M. (2000). A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. Science *288*, 2222–2226.

Mallonee, D.H., White, W.B., and Hylemon, P.B. (1990). Cloning and sequencing of a bile acid-inducible operon from Eubacterium sp. strain VPI 12708. J. Bacteriol. *172*, 7011–7019.

Manwaring, W.H., and Coe, H.C. (1916). Endothelial opsonins. J. Immunol. *1*, 401–408.

Manwaring, W.H., and Fristchen, W. (1923). Study of microbic-tissue affinity by perfusion methods. J. Immunol. *8*, 83–89.

Maolanon, A.R., Madsen, A.S., and Olsen, C.A. (2016). Innovative Strategies for Selective Inhibition of Histone Deacetylases. Cell Chem Biol *23*, 759–768.

Marcobal, A., Yusufaly, T., Higginbottom, S., Snyder, M., Sonnenburg, J.L., and Mias, G.I. (2015). Metabolome progression during early gut microbial colonization of gnotobiotic mice. Sci. Rep. *5*, 11589.

Monteiro, R.C., and Van De Winkel, J.G. (2003). IgA Fc receptors. Annu. Rev. Immunol. *21*, 177–204.

Naugler, W.E., Sakurai, T., Kim, S., Maeda, S., Kim, K., Elsharkawy, A.M., and Karin, M. (2007). Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science *317*, 121–124.

Olszak, T., An, D., Zeissig, S., Vera, M.P., Richter, J., Franke, A., Glickman, J.N., Siebert, R., Baron, R.M., Kasper, D.L., and Blumberg, R.S. (2012). Microbial exposure during early life has persistent effects on natural killer T cell function. Science *336*, 489–493.

Paik, Y.H., Schwabe, R.F., Bataller, R., Russo, M.P., Jobin, C., and Brenner, D.A. (2003). Toll-like receptor 4 mediates inflammatory signaling by bacterial lipopolysaccharide in human hepatic stellate cells. Hepatology *37*, 1043–1055.

Parséus, A., Sommer, N., Sommer, F., Caesar, R., Molinaro, A., Ståhlman, M., Greiner, T.U., Perkins, R., and Bäckhed, F. (2016). Microbiota-induced obesity requires farnesoid X receptor. Gut. Published online January 6, 2016. http://dx.doi.org/10.1136/gutjnl-2015-310283.

Philipp, B. (2011). Bacterial degradation of bile salts. Appl. Microbiol. Biotechnol. *89*, 903–915.

Rath, C.M., and Dorrestein, P.C. (2012). The bacterial chemical repertoire mediates metabolic exchange within gut microbiomes. Curr. Opin. Microbiol. *15*, 147–154.

Ridlon, J.M., Kang, D.J., and Hylemon, P.B. (2006). Bile salt biotransformations by human intestinal bacteria. J. Lipid Res. *47*, 241–259.

Ridlon, J.M., Kang, D.J., and Hylemon, P.B. (2010). Isolation and characterization of a bile acid inducible 7alpha-dehydroxylating operon in Clostridium hylemonae TN271. Anaerobe *16*, 137–146.

Ridlon, J.M., Harris, S.C., Bhowmik, S., Kang, D.J., and Hylemon, P.B. (2016). Consequences of bile salt biotransformations by intestinal bacteria. Gut Microbes *7*, 22–39.

Rivera, C.A., Adegboyega, P., van Rooijen, N., Tagalicud, A., Allman, M., and Wallace, M. (2007). Toll-like receptor-4 signaling and Kupffer cells play pivotal roles in the pathogenesis of non-alcoholic steatohepatitis. J. Hepatol. *47*, 571–579.

Schiff, E.R., Small, N.C., and Dietschy, J.M. (1972). Characterization of the kinetics of the passive and active transport mechanisms for bile acid absorption in the small intestine and colon of the rat. J. Clin. Invest. *51*, 1351–1362.

Schildberg, F.A., Sharpe, A.H., and Turley, S.J. (2015). Hepatic immune regulation by stromal cells. Curr. Opin. Immunol. *32*, 1–6.

Schurich, A., Böttcher, J.P., Burgdorf, S., Penzler, P., Hegenbarth, S., Kern, M., Dolf, A., Endl, E., Schultze, J., Wiertz, E., et al. (2009). Distinct kinetics and dynamics of cross-presentation in liver sinusoidal endothelial cells compared to dendritic cells. Hepatology *50*, 909–919.

Seki, E., De Minicis, S., Osterreicher, C.H., Kluwe, J., Osawa, Y., Brenner, D.A., and Schwabe, R.F. (2007). TLR4 enhances TGF-beta signaling and hepatic fibrosis. Nat. Med. *13*, 1324–1332.

Shiloh, M.U., MacMicking, J.D., Nicholson, S., Brause, J.E., Potter, S., Marino, M., Fang, F., Dinauer, M., and Nathan, C. (1999). Phenotype of mice and macrophages deficient in both phagocyte oxidase and inducible nitric oxide synthase. Immunity *10*, 29–38.

Sinal, C.J., Tohkin, M., Miyata, M., Ward, J.M., Lambert, G., and Gonzalez, F.J. (2000). Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. Cell *102*, 731–744.

Slack, E., Hapfelmeier, S., Stecher, B., Velykoredko, Y., Stoel, M., Lawson, M.A., Geuking, M.B., Beutler, B., Tedder, T.F., Hardt, W.D., et al. (2009). Innate and adaptive immunity cooperate flexibly to maintain host-microbiota mutualism. Science *325*, 617–620.

Spadoni, I., Zagato, E., Bertocchi, A., Paolinelli, R., Hot, E., Di Sabatino, A., Caprioli, F., Bottiglieri, L., Oldani, A., Viale, G., et al. (2015). A gut-vascular barrier controls the systemic dissemination of bacteria. Science *350*, 830–834.

Stappenbeck, T.S., Hooper, L.V., and Gordon, J.I. (2002). Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. Proc. Natl. Acad. Sci. USA *99*, 15451–15455.

Su, G.L., Klein, R.D., Aminlari, A., Zhang, H.Y., Steinstraesser, L., Alarcon, W.H., Remick, D.G., and Wang, S.C. (2000). Kupffer cell activation by lipopolysaccharide in rats: role for lipopolysaccharide binding protein and toll-like receptor 4. Hepatology *31*, 932–936.

Taur, Y., and Pamer, E.G. (2016). Microbiome mediation of infections in the cancer setting. Genome Med. *8*, 40.

Trauner, M., and Boyer, J.L. (2003). Bile salt transporters: molecular characterization, function, and regulation. Physiol. Rev. *83*, 633–671.

Uesugi, T., Froh, M., Arteel, G.E., Bradford, B.U., and Thurman, R.G. (2001). Toll-like receptor 4 is involved in the mechanism of early alcohol-induced liver injury in mice. Hepatology *34*, 101–108.

Uhrig, A., Banafsche, R., Kremer, M., Hegenbarth, S., Hamann, A., Neurath, M., Gerken, G., Limmer, A., and Knolle, P.A. (2005). Development and functional consequences of LPS tolerance in sinusoidal endothelial cells of the liver. J. Leukoc. Biol. *77*, 626–633.

Venkatesh, M., Mukherjee, S., Wang, H., Li, H., Sun, K., Benechet, A.P., Qiu, Z., Maher, L., Redinbo, M.R., Phillips, R.S., et al. (2014). Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like receptor 4. Immunity *41*, 296–310.

Wang, L., Fouts, D.E., Stärkel, P., Hartmann, P., Chen, P., Llorente, C., DePew, J., Moncera, K., Ho, S.B., Brenner, D.A., et al. (2016). Intestinal REG3 Lectins Protect against Alcoholic Steatohepatitis by Reducing Mucosa-Associated Microbiota and Preventing Bacterial Translocation. Cell Host Microbe *19*, 227–239.

Wardlaw, A.C., and Howard, J.G. (1959). A comparative survey of the phagocytosis of different species of bacteria by Kupffer cells; perfusion studies with the isolated rat liver. Br. J. Exp. Pathol. *40*, 113–117.

Wells, J.E., and Hylemon, P.B. (2000). Identification and characterization of a bile acid 7alpha-dehydroxylation operon in Clostridium sp. strain TO-931, a highly active 7alpha-dehydroxylating strain isolated from human feces. Appl. Environ. Microbiol. *66*, 1107–1113.

Wingender, G., Stepniak, D., Krebs, P., Lin, L., McBride, S., Wei, B., Braun, J., Mazmanian, S.K., and Kronenberg, M. (2012). Intestinal microbes affect phenotypes and functions of invariant natural killer T cells in mice. Gastroenterology *143*, 418–428.

Wöhler, F., and Frerichs, F.T. (1848). Ueber die Veränderungen, welche namentlich organische Stoffe bei ihrem Uebergang in den Harn erleiden. Annalen der Chemie und Parmacie *65*, 335–349.

Wolf, M.J., Adili, A., Piotrowitz, K., Abdullah, Z., Boege, Y., Stemmer, K., Ringelhan, M., Simonavicius, N., Egger, M., Wohlleber, D., et al. (2014). Metabolic activation of intrahepatic CD8+ T cells and NKT cells causes nonalcoholic steatohepatitis and liver cancer via cross-talk with hepatocytes. Cancer Cell *26*, 549–564.

Wong, C.H., Jenne, C.N., Petri, B., Chrobok, N.L., and Kubes, P. (2013). Nucleation of platelets with blood-borne pathogens on Kupffer cells precedes other innate immunity and contributes to bacterial clearance. Nat. Immunol. *14*, 785–792.

Xiang, B., Zhang, G., Guo, L., Li, X.A., Morris, A.J., Daugherty, A., Whiteheart, S.W., Smyth, S.S., and Li, Z. (2013). Platelets protect from septic shock by inhibiting macrophage-dependent inflammation via the cyclooxygenase 1 signalling pathway. Nat. Commun. *4*, 2657.

Yu, L.X., Yan, H.X., Liu, Q., Yang, W., Wu, H.P., Dong, W., Tang, L., Lin, Y., He, Y.Q., Zou, S.S., et al. (2010). Endotoxin accumulation prevents carcinogen-induced apoptosis and promotes liver tumorigenesis in rodents. Hepatology *52*, 1322–1333.

Zhang, W., Daly, C.G., Mitchell, D., and Curtis, B. (2013). Incidence and magnitude of bacteraemia caused by flossing and by scaling and root planing. J. Clin. Periodontol. *40*, 41–52.

Zhou, H., and Hylemon, P.B. (2014). Bile acids are nutrient signaling hormones. Steroids *86*, 62–68.