1 Organizational Principles of the Liver

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PRINCIPLES OF LIVER STRUCTURE AND FUNCTION

The liver is the largest organ of the mammalian body and has a highly versatile and complex function. Its specialized role is shown by the fact that, despite intense efforts, the activity of the liver cannot be replaced by artificial equipment. The liver participates in the maintenance of the organism's homeostasis as an active, bidirectional biofilter. It is classed as bidirectional because it filters the portal blood that transports nutritional and toxic compounds from the environment through the gastrointestinal tract and also filters the systemic blood (the body's own products, e.g. bilirubin), providing the only channel of the body, the biliary system, through which non-water-soluble substances can be removed. It is classed as an active filter because it rapidly metabolizes most nutritional compounds and neutralizes and prepares for removal toxic exogenous (xenobiotics) and endogenous (worn out) materials. Because of these major functions the liver is constantly exposed to intense microbiological and antigenic stimuli which require function of the innate and adaptive immune systems. These diversified functions are executed by a structurally complex, multicellular tissue with a unique angioarchitecture, and by the combined and integrated activities of the participants.

There are only two unique cell types in the liver – hepatocytes and biliary cells (or cholangiocytes). The hepatocytes are "the most valuable" parenchymal cells of the hepatic tissue. They do not constitute a homogeneous cell population. They are highly polarized cells (i.e. molecular specializations of the various surface membranes, including receptors, pumps, transport channels and carrier proteins) and their functions and to a certain extent morphology depend on their location in the parenchyma. This polarization makes the hepatocytes the logical center of the liver. In addition, they perform the most complex metabolic tasks of the mammalian organism.

The cholangiocytes form the channels that constitute the biliary system, which drains the parenchyma and guarantees the permanent flow of the bile, a highly toxic solution. Cholangiocytes also modify the composition of the bile and, in case of adverse conditions, can participate in repair mechanisms. These liver cells could not carry out their specific functions, of course, without the support of several "communal" cell types, which are highly adapted to the special function and architecture of the liver. The endothelial cells of the parenchyma have a unique fenestrated structure and various different subpopulations can be distinguished. There are several subpopulations of hepatic myofibroblasts as well. In addition to their mechanical functions the myofibroblasts can store special substances (e.g. vitamin A in stellate cells) and are a major source of growth factors and cytokines. The Kupffer cells are the resident macrophages in the liver. In addition to filtering the blood, they perform their traditional immunregulatory function. The presence of almost all subtypes of lymphocytes and dendritic cells makes the liver the largest organ of the immune system. The mesothelial cells of the Glisson capsule are, beside their mechanical function, an important source of lymph production and can contribute to the generation of other hepatic cell types. The features of the hepatic extracellular matrix are unique. The components of the basement membrane are present around the sinusoids in an "unstructured" fashion, and cannot be detected by electron microscope, yet they can perform certain functions.

Another fundamental feature of liver organization is its unique vascular pattern. Two afferent vessels supply blood to the liver: the portal vein and the hepatic artery. The blood of the portal vein, having already "drained" the stomach, gut, pancreas, and spleen, is reduced in oxygen and pressure, and is

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enriched in nutrients and toxic materials absorbed from the alimentary tract and in viscerally generated hormones and growth factors. The arterial blood of the hepatic artery has systemic levels of oxygen, pressure, and composition. The major function of the hepatic artery is to supply the peribiliary vascular plexus, the portal tract interstitium, the hepatic capsule, and the vasa vasorum of major vessels. In some species, the hepatic artery forms anastomosis with the branches of the portal vein, but even then this blood also ends up in the sinusoids. The blood of the liver is collected by one efferent draining system, the hepatic or "central" veins, which reach the systemic circulation via the inferior vena cava. The sinusoids form a very special vascular system, which is interposed between the afferent and efferent vessels. The large number and capacity of the sinusoids and the special arrangement of the supplying vessels provide a large volume of blood at a high flow rate via the large vessels with high compliance and capacity. At the same time the sinusoids are perfused with blood at low pressure and flow rate. These arrangements (i.e. low flow, specifically fenestrated (perforated) endothelial cells, and the lack of the structured basement membrane) provide an especially efficient communication between the blood and hepatocytes. This is well illustrated by the pathological condition of liver cirrhosis, when the changes in hemodynamic condition (i.e. the "capillarization" of the sinusoids) disrupts this communication, resulting in severe dysfunction of the liver.

Bile acids and their enterohepatic circulation are another good example of the cumulating functions. The bile acids are synthetized in the hepatocytes by a complex biochemical process that requires 16 different enzymes, which are further modified by the gut microbiota. The primary physiological function of the bile acids is to convert lipid bilayers into micelles. This makes possible the excretion of important waste products from the blood. The bile acids also emulsify elements of the food in the gut and aid their absorption. In addition, bile acids act as signaling molecules, synchronizing the cooperation of the liver and gut.

The different types of cells and vessels mentioned above can operate only if they are organized in a well "designed" structure. The most widely studied and analyzed morphological and functional unit or module of the liver is the hepatic lobule. The popularity of this structure for studies can be partly explained by the fact that lobules are outlined nicely in some species (pig, camel, bear) by connective tissue septa, and can therefore be easily recognized on the two-dimensional histological sections commonly used in structural studies. The idealized lobule has a polygonal (usually hexagonal) shape. The terminal branch of the hepatic vein (central vein) is in the center of the lobule while the corners are occupied by the "portal triads." The components of the triad are the interlobular bile ducts and the terminal branches of the portal vein and hepatic artery. The blood carried by these afferent vessels is distributed by the inlet venules and arteries along the virtual "vascular septa." This vascular frame is filled up columns (or sheets in three-dimensional space) of the hepatocytes constructed as "plates" arranged in a radial fashion. The hepatic plates are separated by the similarly distributed sinusoids. The blood runs in a centripetal direction from the vascular septa to the central vein. The vascular septa secure the mixing of the portal venous and arterial blood and the more-or-less equal supply to the sinuses. The bile produced by the hepatocytes runs in a centrifugal direction in the bile canalicules formed by neighboring hepatocytes and is collected by the interlobular bile ducts of the portal triads. There is thus a countercurrent between the flow of the blood and bile at lobular level.

FUNCTIONAL ANATOMY OF THE LIVER

Macroanatomy

The liver is a continuous sponge-like parenchymal mass penetrated by tunnels (lacunae) that contain the interdigitating networks of afferent and efferent vessels [1]. The adult human liver weighs from 1300 to 1700 g, depending on sex and body size. It is relatively small compared to other species (2% of the body weight) – in rat and mouse the liver is 4–5% of the body.

In most mammalian species the liver is multilobed, the individual lobes reflecting the distribution of the major branches of afferent and efferent blood vessels. In contrast, the human liver parenchyma is fused into a continuous parenchymal mass with two major lobes, right and left, delineated only by being supplied and drained by separate first- and second-order branches of the portal and hepatic veins. Right and left lobes are topographically separated by the remnants of the embryonic umbilical vein (the falciform ligament), but this landmark does not locate the true anatomic division. Anatomically, the medial segment of the left lobe is located to the right side of the falciform ligament, centered on the anterior branches of the left portal vein. Interdigitation of first- and second-order branches of the portal and hepatic veins produces eight macrovascular parenchymal segments centered on large portal veins and separated by large hepatic veins [2]. Hemodynamic watersheds or fissures separating afferent and efferent macrovascular segments permit the surgical resection of individual or adjacent segments.

Liver transplantation and surgery has reached such a complexity, however, that the traditional eight-segment scheme is no longer sufficient. Detailed histological and imaging investigations have revealed that the number of second-order branches given off by the left and right portal veins is much higher, and the mean of their number is 20, leading to the "1–2–20" concept of portal venous segmentation [3]. The recognition of the watershed septa between the variable actual segments is helped by intraoperative imaging techniques in real operative situations.

Microanatomy

Normal liver function requires the unique arrangement of basic components of hepatic tissue: portal vein, hepatic artery, bile duct, hepatic vein, and hepatocytes. These form in two-dimensional sheets the above-mentioned hepatic (classical of Kiernan's) lobules. Profiles of portal tracts and hepatic veins of various sizes are a prominent feature of liver histology [4–6]. Smaller branches of the afferent and efferent vessels (together with their stromal components) predominate in tissue sections taken from peripheral, subcapsular locations, whereas tissue sections taken from more proximal areas nearer to the hilum contain larger vascular structures [6]. These vascular/stromal elements are contained in tunnels (lacunae) that penetrate the

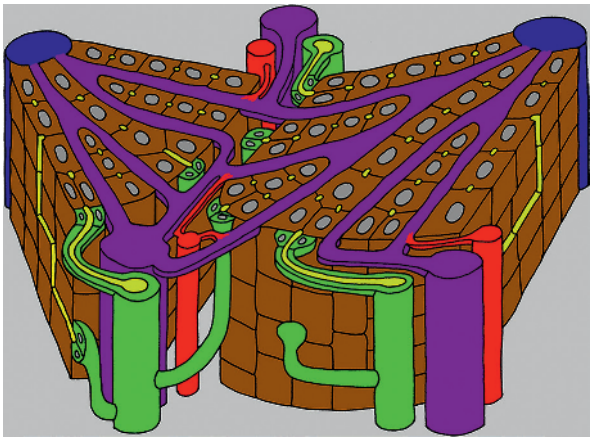

Figure 1.1 Schematic drawing of the organization of blood vessels (arteries, red; portal veins, purple; central veins, blue; bile ducts, green; lumen of the biliary channels, including bile canaliculi, yellow) in two adjacent lobules of human liver. One sixth of a lobule is visible on the right and one third of a lobule is visible on the left. A terminal portal venule, arteriole, and bile ductule (canal of Hering) are present in the vascular septum between the lobules. The arteriole is connected directly to the sinusoids or enters the inlet venule. Bile is drained over the whole surface of the lobule. Arterioles and bile ductules are not present in the vascular septum in rodents. The bile is drained through canals of Hering connected to hepatocytes of the limiting plate. The arterioles anastomose with the portal system at higher level as well. Courtesy of Sandor Paku, Semmelweis University, Budapest, Hungary.

parenchymal mass [4]. The hepatocytes arranged in plates fill in the space between the portal tracts and hepatic veins (Figure 1.1). The hepatic plates form brick-like walls (muralia) of hepatocytes one cell (one brick) thick. The first hepatocytes of the hepatic plates form a virtual barrier between the periportal connective tissue and the liver parenchyma called a limiting plate.

The blood vessels and their investments of connective tissue provide the soft, spongy liver with its major structural support, or "skeleton." Larger afferent vessels, portal veins, and hepatic arteries are contained together with bile ducts in connective tissue – the portal tracts – which are continuous with the mesenchymal components of the liver's mesothelium-covered surface capsule (Glisson's capsule). Portal tracts also contain lymphatic vessels, nerves, and varying populations of other types of cells, such as macrophages, immunocytes, myofibroblasts, and possibly hematopoietic stem cells (see [7] and references therein). The collagenous investment of the efferent vessels is less robust and lacks large numbers of adventitious cells.

The hepatic artery is distributed to the tissues of portal tracts, the liver capsule, and the walls of large vessels [4–6]. In portal tracts arterial branches form a capillary network (the peribiliary plexus) arborized around bile ducts [8, 9]. Efferent twigs from the peribiliary plexus empty into adjacent portal veins in rat and mouse but not in human and hamster [10]. The portal vein supplies blood to the parenchymal mass through the so-called inlet venules [9, 11].

In histological sections of mammalian liver, afferent and efferent vessels interdigitate regularly in an approximate ratio of 5–6 portal tracts for each profile of a hepatic vein, to form a pattern of cross-sections of portal tracts and hepatic veins separated by parenchyma [5, 6]. Most of the cross-sectioned portal tracts contain preterminal hepatic venules. These vessels represent the seventh- to tenth-order branches from the hilar portal vein in large mammals, such as humans. These small portal tracts and hepatic (central) veins penetrate the parenchyma in nearly parallel orientations about 0.5–1.0 mm apart. The portal inlet venules are very short vessels with no smooth muscle in their walls. They branch from preterminal and terminal venules at points on the circumference of the lobules at about 120 radial degrees (triradial branching) and penetrate the parenchyma together with terminal arteriolar branches approximately perpendicular to and midway between two adjacent terminal hepatic venules [5, 6]. During their course through the parenchyma portal inlet venules break up completely into sinusoids, which are oriented more or less perpendicularly to the veins. Because they are hardly larger than sinusoids, the inlet venules are not conspicuous in humans and other mammals that lack a definite connective sheath around them. However, in adult swine their course through the parenchyma is clearly marked by connective tissue.

Capillary-size sinusoids occupy the smallest and most numerous tunnels (lacunae) in the parenchymal mass [4]. Unlike capillaries elsewhere, liver sinusoids are composed of endothelial cells that are penetrated by holes (fenestrae) and lack a structured basal membrane [12], features that allow free egress of the fluid components and solutes of the perfusing blood. For example, tagged albumin has access to a space in the liver that is about 48% larger than the sinusoidal volume, in contrast to other tissues in which capillary space and albumin space are nearly the same [13]. In favorably oriented histological sections, more or less parallel, longitudinal profiles of sinusoids alternate with hepatic plates [14]. A narrow cleft, called the space of Disse, separates sinusoids from hepatocytes located in adjacent hepatic plates [12, 15]. At their proximal (portal venous) ends, sinusoids are narrow and somewhat tortuous, whereas their middle and distal (hepatic venous) portions are larger and straighter [9, 16, 17]. Sinusoids and hepatic plates are disposed radially around the draining hepatic veins and extend directly to the supplying inlet venules [17].

Three-dimensional reconstruction of the interlobular zone revealed the existence of a small vessel in this plane, the vascular septum, that serves as a starting pool for intralobular sinusoids. This is a hemodynamic barrier, a "watershed" between the two neighboring lobules. This "interlobular" septum contains connective tissue matrix in pig, camel, bear, etc. and outlines the lobules nicely; it also exists in a rudimentary form in human liver [18].

The bile – the excretion product of the hepatocytes – is collected and transported in bile canaliculi, which are formed by the apical sides of two adjacent hepatocytes in the hepatic plate. The network of canaliculi is drained into the interlobular bile ducts through interface structures called canals of Hering. These are intermediary structures constructed partly by hepatocytes or cholangiocytes (Figure 1.2). Since these structures are the primary candidates to harbor the hepatic stem cell compartment, they are the subject of intensive investigations [19]. The distribution of canals of Hering shows variation among different species. They are characterized by a distinct ($EMA^-/CD56^+/CD133^+$) immunophenotype in humans, leave the periportal space and spread into the parenchyma along the rudimentary interlobular septa, and thus do not enter into the hepatic lobule [18].

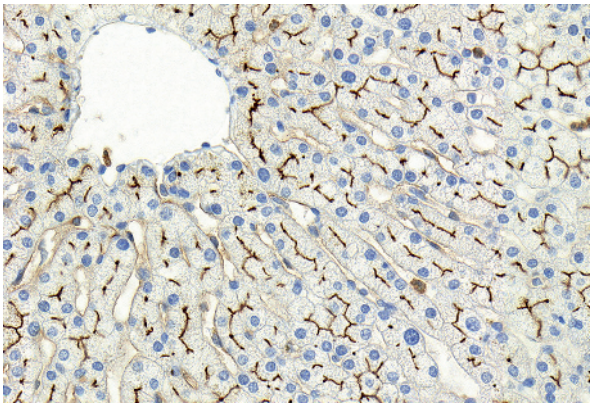

Figure 1.2 Normal human liver stained for CD10. The hepatocytes form trabeculae, 1–2 cells thick, radiating from a central vein. CD10 is expressed on the canalicular domain, indicating the polarization of the cells. Courtesy of Sandor Paku, Semmelweis University, Budapest, Hungary.

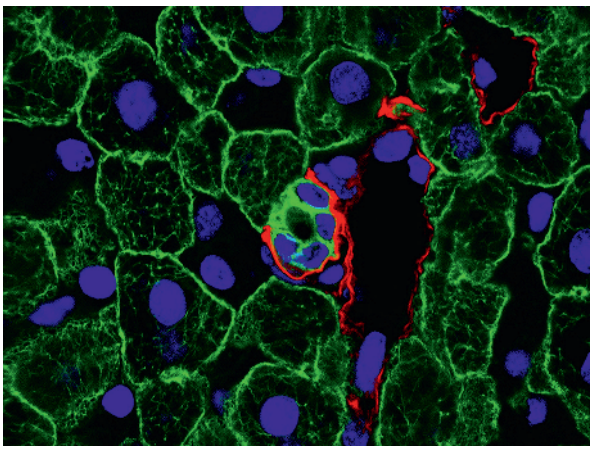

Figure 1.3 Normal rat liver stained for pankeratin (green) and laminin (red), the nuclei are labeled by TOTO (blue). There is a cross-section of a canal of Hering beside a small portal vein. The cytoplasm of the cholangiocytes is strongly positive for keratin and these cells are outlined by laminin (basement membrane) but basement membrane is absent at the pole where the ductile is connected to an adjacent hepatocyte. Courtesy of Sandor Paku, Semmelweis University, Budapest, Hungary.

The interlobular bile ducts are lined by a single layer of cuboidal cholangiocytes (Figure 1.3). They anastomose and unite larger septal and hilar branches. The connective tissue around the largest biliary branches contain peribiliary glands which also secrete into the biliary tract (Figure 1.4).

Teutsch and coworkers [20, 21] analyzed serial sections of rat and human livers to reconstruct the three-dimensional structure of hepatic tissue. Although there were differences between the two species, the basic arrangement was similar. The reconstruction revealed primary "modules," which constructed a more complex "secondary" module. The integration was based on a common drainage by branches of the hepatic veins and supplying portal veins, and the modules were covered by continuous vascular septa. The primary modules correspond to the two-dimensional hepatic lobules. Quite a substantial variation in the shape and size of the modules was found, which provides morphogenetic plasticity to construct the whole organ. This

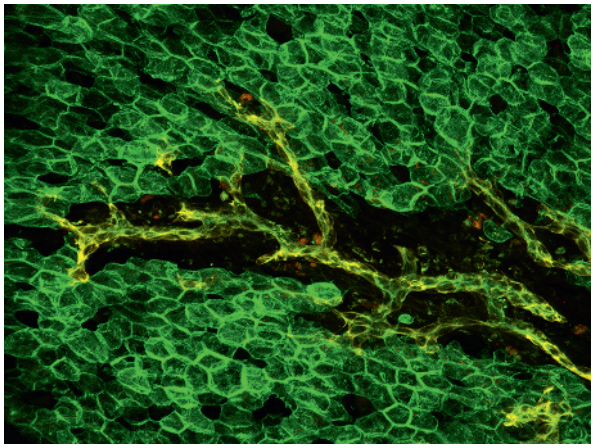

Figure 1.4 Normal human liver stained for pankeratin (green) and keratin 7 (red). The dark lane in the center represents an interlobular vascular septum. The double positive (yellow) biliary ductules have several connections with the limiting hepatocytes but do not enter into the lobules. Courtesy of Sandor Paku, Semmelweis University, Budapest, Hungary.

modular arrangement can improve the interpretation of lesions, especially in pathologically altered livers, but it is certainly not easy to transform the two-dimensional observations into three-dimensional space.

Functional unit of the liver

The concept of the primary functional unit of the liver has been the subject of debate for more than 350 years since its description by Wepfler in 1664 [22]. The first and most widely accepted traditional unit of the liver is "Kiernan's lobule" [23], as described earlier. This is the efferent microvascular segment, being the smallest unit of parenchyma that is drained of blood by a single efferent (terminal hepatic or central) vein. It is quite easy to identify it, especially in species where they are outlined by connective tissue. The major criticism of the concept is that the terminal afferent vessels through the vascular septa contribute to the blood supply of adjacent lobules, and therefore the lobule cannot be a "basic functional unit." Rappaport defined the basic unit as the compartment of the hepatic parenchyma supplied with blood by a single terminal portal vein and called this unit the "liver acinus" [24]. Now we know that this unit is also supplied by a single terminal branch of the hepatic artery. The simple acinus is a parenchymal mass around a portal tract and it is drained by more hepatic venules. The acinus is subdivided into three zones, based on the distance from the portal vein. The distribution of these areas fits to the functional zonality of the hepatic parenchyma. Pathological lesions (e.g. steatosis or necrosis) also often follow this zonal pattern, which made this unit attractive. However, this zonality did not correspond perfectly to the distribution of enzyme activities and the hepatic modules described by Teutsch [20, 21] were also not compatible with the concept of the acinus.

Matsumoto and his colleagues [6] investigated the angioarchitecture of the human liver on thousands of serial sections, distinguishing conducting and parenchymal portions of the portal venous tree. A cone-shaped parenchymal portion (primary lobule) was defined which was supplied by a terminal portal venule.

The circulatory network of this unit was named the hepatic microcirculatory subunit (HMS). Ekataksin and Wake [25] showed that the afferent microvascular segment is also the smallest unit of parenchyma drained of bile by terminal bile ducts [25], demonstrating that this hemodynamic segment is also the smallest excretory unit of parenchyma. The compartment of the hepatic parenchyma associated with an HMS contains all of the elementary structures of the hepatic tissue and may represent the elementary functional and morphological unit of the liver. It was named cholehepaton. The cholehepaton is also the smallest bile–blood unit and conforms with the principle of countercurrent flow. The cholehepaton has no anatomical or structural borders, and cannot be recognized on histological sections. It is defined by its function and mostly corresponds to Matsumoto's primary lobules. Six of these Matsumoto's primary lobules constitute the secondary lobule, which is almost identical with the classical Kiernan's lobule. This long and complicated detour returned us to the classical lobule, which is widely used today to analyze reactions of the hepatic tissue.

However, it is worth remembering that there are other types of hepatic functional units and some workers contend that the hepatic tissue is an indivisible continuum that has no definable units [26].

Liver hemodynamics

The hepatic vasculature is characterized by high capacity, high compliance, and low resistance [27]. Blood vessels comprise about 22% of the liver's mass/volume [13] and the liver contains about 12% of the body's total blood volume under physiological conditions [27], a sizeable fraction of which can be expelled by contraction of larger vessels by sympathetic nerve stimulation. In other words, the liver is a blood reservoir. The pressure of portal venous blood is reduced as the major afferent vessels dichotomize through the parenchyma, from about 130 mmH_2O in the extrahepatic portal vein to about 60 mmH_2O in the preterminal portal veins of the exteriorized liver of the anesthetized rat, amounting to about 60% of the total transhepatic pressure gradient [13]. A similar portal pressure gradient has been found in humans [27]. Blood flow through the liver amounts to about 1500–2000 mL min^{-1} in adult humans, about 25% of the resting cardiac output [27]. About 25% of the total liver blood flow is derived from the hepatic artery at prevailing arterial pressure and oxygenation. The portal venous blood (about 75% of total liver blood flow) arrives at the liver partially depleted of oxygen and at a reduced pressure as a consequence of having already perfused the splanchnic viscera.

In aggregate, sinusoids comprise about 60% of the liver's vascular volume, or about 13% of the total liver mass/volume [13]. A significant decrease in blood pressure occurs in sinusoids (about 40% of the transhepatic pressure gradient), the pressure declining to about 25 mmH_2O in terminal hepatic veins of exteriorized liver of anesthetized rats [28]; the pressure gradient in the short sinusoids is especially steep. Blood pressure in the inferior vena cava approximates that in the terminal hepatic vein [27]. Consequently, although flow of blood through sinusoids faces little resistance, it is slow and somewhat intermittent and is assisted by negative pressure produced by respiratory expiration [27]. Possible mechanisms of regulating blood flow within sinusoids are controversial. The sinusoidal circulation is regulated by a sphincter in the terminal arterioles [29]. In addition, hepatic stellate cells are the pericytes of the sinusoids and are the principal regulators of sinusoidal blood flow. They have receptors for molecular mediators regulating contraction and are closely associated with nerve endings, indicating neurogenic influence [30]. Sinusoids appear to have limited contractile ability, possibly produced by contraction of encircling stellate cells (pericytes) [29, 31]. Studies of the exteriorized liver of rodents suggest that sinusoidal flow may be regulated at both inlet and outlet levels [32], although other similar studies have not detected sphincters at either point [28]. However, sinusoidal flow is strongly affected by post-sinusoidal resistance [27].

THE CELLS OF THE LIVER

Hepatocytes

Hepatocytes are commonly denoted as "parenchymal cells" and the other cells of the liver tissue matrix as "non-parenchymal cells." This convention is somewhat artificial since hepatocytes alone are not competent to perform all essential hepatic functions, and several types of cells in the liver tissue matrix function as an integrated community to carry out conjointly the multiplicity of hepatic functions. Functional integration of this cellular community is accomplished by several communication mechanisms, including signaling networks involving numerous cytokines and chemokines, and by direct transfer of small molecules through gap junctions [33]. Hepatocytes, responsible for most of the synthetic and many of the metabolic functions of the liver (see Chapters 23 and 24), are large polygonal cells (averaging about 25–30 μm in cross-section and 5000–6000 μm^3 in volume [34]). They are the most numerous cells in the liver parenchyma; the adult human liver probably contains about 10^{11} hepatocytes, representing about 60% of all cells in the parenchymal matrix and comprising about 80% of its mass/volume [15]. Hepatocytes are shaped as complex rhomboids with several distinct surfaces [34]. They are polarized by molecular specializations of their various surface membranes in the forms of receptors, pumps, transport channels, and carrier proteins (see Chapters 5 and 6) that comprise three functionally distinct domains (see Chapter 6): the basolateral domain, the lateral domain, and the canalicular domain.

- *Basolateral domain*: The basolateral (or sinusoidal) domain constitutes about 35% of the total hepatocyte surface facing the sinusoids. The area of this surface is greatly amplified by the folding of the plasma membrane to form innumerable microvilli that extend into the space of Disse [34]. This membrane is equipped for extraction of a great variety of molecules from the blood and for the simultaneous secretion into the blood of other molecules that have been modified or synthesized by hepatocytes. The cell matrix adhesion molecules are also present on this side of the hepatocyte. Although there is no structured basement membrane between the hepatocytes and sinusoidal endothelial cells, the lack of integrin-linked kinase 1 (Ilk1) results in disruption of the normal structure [35], supporting the necessity of hepatocyte–matrix interaction. About 50% of the total hepatocyte surface faces adjacent hepatocytes [34].

- *Lateral domain*: The plasma membranes of these intercellular surfaces are mostly flat lateral domain, which is the least complex. This surface contains intercellular adhesion complexes (tight junctions, intermediate junctions, and desmosomes) that pin together the adjacent hepatocytes, form a permeability barrier between the peri-sinusoidal space of Disse and bile canaliculi, and include gap junctions that allow communication between adjacent hepatocytes by transfer of small molecules.
- *Canalicular domain*: Infoldings of the lateral surfaces create bile canaliculi, which comprise about 13% of the total hepatocyte surface. This is termed the canalicular domain [34]. It is also amplified by microvilli and modified for bile excretion. The canalicular surface is confined by strong junctional complexes. Bile canaliculi form a belt-like extracellular space (about 1 μm in diameter) that is continuous along the lengths of the hepatic plates, connecting at the portal ends with bile ducts. The molecular mechanisms responsible for the polarity of the canalicular domain are well characterized. Hepatocyte nuclear factor 4 (Hnf4α) is the master regulator of morphological and functional hepatocyte differentiation [36], but several other factors, such as liver kinase B1 (Lkb1) [37], vacuolar sorting protein 33b (Vps33b) [38], and claudin-15 [39], are also required for the polarization.

As befits their numerous metabolic functions, hepatocytes contain a complex array of mitochondria (~1700 per cell on average), peroxisomes (~370 per cell), lysosomes (~250 per cell), Golgi complexes (~50 per cell), aggregates of rough and smooth endoplasmic reticulum (~15% of cell volume), and numerous microtubules/microfilaments [34].

Polyploidization is another unique feature of the hepatocytes. This is the result of defective cytokinesis, which seems to be regulated by the insulin–Akt signaling pathway [40]. The extent of ploidy increases with age. The presence of aneuploid hepatocytes in the normal liver is also well established [41]. The exact function of this process is still unknown but it is thought to help in adaptation to chronic injury.

Hepatic plates and adjacent sinusoids form associations that are structurally similar in all parts of the liver. Various liver cells show numerical, structural, and functional heterogeneities related to their location along the afferent–efferent axis of hepatic plates and sinusoids. Among the structural differences are ploidy variations in hepatocytes; in adult mammals hepatocytes located at the portal ends of hepatic plates are diploid, whereas cells of higher ploidy are located further downstream [42]. Gap junctions containing connexin 26 are more numerous on portal hepatocytes, whereas junctions containing connexin 32 are distributed on hepatocytes in all parts of hepatic plates [43]. These variations in structure and cellular composition are associated with functional differences among hepatocytes located at different points along the afferent–efferent axis of plates and sinusoids. Rappaport divided the portal-hepatic (afferent–efferent) lengths of hepatic plates into three arbitrary zones (termed I, II, and III). Hepatocytes located in these zones differ in their functional capabilities and susceptibilities to pathological damage [24], and pathways performing opposing functions follow an inverse distribution along the portocentral axis. This is strikingly exemplified by regional differences in carbohydrate metabolism (gluconeogenesis and glycogen storage by periportal hepatocytes; glycolysis by perihepatic vein hepatocytes). The ammonia and fatty acid metabolisms are also zonally distributed. Zone 1 hepatocytes are engaged in urea production and β-oxidation of fatty acids, while zone 3 hepatocytes remove nitrogen by glutamine synthetase and perform lipogenesis. The metabolism of xenobiotics is more prominent in the pericentral hepatocytes. Recent research has shown that many liver functions are dispersed heterogeneously, with dispersed functions often acting as integrated parts of coordinate metabolic systems [44].

Zonation of liver functions was thought to be related to sinusoidal hemodynamics, which produced gradients in blood-borne substances available to hepatocytes and other cells of the parenchymal matrix [44]. Recent evidence gained mostly from experiments with genetically engineered mice proved that the Wnt/β-catenin pathway is the master regulator of hepatocytic zonation, but the interaction with lymphoid enhancer factor 4 (Lef4) and HNF4α is also critical [45]. Hepatocytes and other cells located at afferent and efferent ends of hepatic plates are subjected to different microenvironmental conditions. Certain molecules are largely extracted by the first hepatocytes that encounter the perfusing blood, lowering their concentration downstream. For example, oxygen levels in the blood at afferent and efferent ends of sinusoids differ greatly because oxygen is efficiently extracted by hepatocytes located at the afferent ends of hepatic plates, exposing downstream hepatocytes to relatively hypoxic conditions; the oxygen gradient alone can explain much of the heterogeneity of hepatocyte function related to position in plates [46]. Other molecules modified or produced by upstream hepatocytes are excreted into the sinusoidal blood and may be removed by hepatocytes located further downstream. The complex interplay of metabolite concentration in the perfusing blood, coupled with extraction, modification, secretion, re-extraction, and further modification, influence the metabolic events that occur in individual cells and define unequal parenchymal territories that produce zonal variations in different physiological capabilities and pathological susceptibilities [44]. Disruption of metabolic zonation has severe consequences.

Physiological turnover of hepatocytes occurs slowly. They have a lifespan of about 400 days in an adult steady-state hepatocyte population, about 0.025% of which typically are cycling [32] and the remainder rest in G0 phase. Although the hepatocytes are ready to re-enter the cell cycle upon injury, this capacity decreases with age. There are, however nonresolved opposing opinions about the replacement of lost hepatocytes during homeostatic conditions. The contribution of hematopoietic cells to liver maintenance was a very popular idea 20 years ago. Repopulation of bone marrow-derived cells (macrophages, myofibroblasts) in the liver is evident in recipients of liver transplants, in which these types of liver cells are replaced with cells of the host genotype [47]. In contrast, hepatocytes are not generated from bone marrow cells in significant numbers under either circumstance [47].

However, the ancient and very attractive theory of "streaming liver" keeps returning. It was originally proposed by Zajicek and colleagues [48] that periportal hepatocytes have enhanced replicative potential and the progeny of these cells spread under

homeostatic condition in a portal–central direction. Although few studies applying lineage-tracing methodology have supported this notion, most of the studies have ruled out this option. Most recently, Axin2$^+$ hepatocytes [49] abutting hepatic veins as well as hybrid periportal hepatocytes [50] have been reported to have selective growth advantage. Based on these results a bidirectional streaming hypothesis, "somehow like ocean water entering into the delta of the Amazon River at high tide" has been proposed [51]. This issue will no doubt be resolved in the near future.

Cholangiocytes

Cholangiocytes, or biliary epithelial cells, comprise much less than 1% of the total number of cells in the liver parenchyma, since most are located in bile ducts in portal tracts [52]. Only the smallest bile ducts penetrate the parenchymal mass in the company of terminal portal veins, where they connect with bile canaliculi in hepatic plates. The points of connection of ducts with hepatic plates are defined by tubular structures called the canals of Hering, which are composed of both cholangiocytes and hepatocytes [53]. They are also the location of liver epithelial stem cells that can differentiate into both hepatocytes and cholangiocytes [54, 55]. Larger bile ducts contain cholangiocytes that rest on a basal membrane and vary in number and size in proportion to duct size [56]. The cholangiocytes are also polarized cells, with an apical (luminal) and a basolateral domain. Their luminal surface membranes are expanded by microvilli and a single primary cilium, which is a sensor for mechanical, osmolar, and chemical stress [57]. Although they contain fewer mitochondria and sparser endoplasmic reticulum than do hepatocytes, cholangiocytes in intrahepatic bile ducts, together with the network of capillaries that surrounds them (the peribiliary plexus), form a metabolic unit that modifies the composition of canalicular bile [58].

The biliary tree is not just a draining pipe and 70–90% of the bile volume is produced by the cholangiocytes. They change the composition of the bile by secretion and absorption. The main secretory product is bicarbonate, while they absorb bile acids, glucose, and glutamate. The cholangiocytes also play an important immunoregulatory role. They are in the first line of defense against microbial components of the biliary tract, xenobiotics, and foreign antigens. The cholangiocytes tackle these challenges by maintaining immunotolerance. They are equipped with pathogen recognition receptors (PRR), all members of the toll-like receptors (TLR1–10), as well as related signaling molecules. Cholangiocytes produce antibacterial products (e.g. defensins, lactoferrin, lysozyme, and transport IgA) into the lumen and express MHC class I and II molecules and antigen presenting cells [59]. It is not surprising, therefore, that the biliary tree is often affected by immunological disorders, such as primary sclerosing cholangitis, primary biliary cholangitis, and graft-versus-host disease. The cholangiocytes are slow turnover cells, but under special condition they can also participate in the regeneration of liver parenchyma (see later).

Endothelial cells

Endothelial cells of sinusoids comprise about 3% of the parenchymal mass/volume [15] and probably number about 3×10^{10} in an adult human liver. Liver sinusoidal endothelial cells are highly specialized endothelial cells with peculiar morphology and function. They are extremely thin in normal liver, 150–170 nm across, and this attenuated cytoplasm is fenestrated. The diameter of these "perforations" ranges from 50 to 200 nm, and they are clustered together in groups to form "sieve plates" [60]. The fenestrae are dynamic structures, and their actual diameter is influenced by blood pressure, composition of extracellular matrix, hormones, etc. The porosity of the sinusoidal endothelial cells is polarized, and the fenestrae are more numerous in the centrilobular zone. The maintenance of the fenestration requires paracrine and autorcrine signals, which are mostly provided by hepatocytes and stellate cells [61]. Surprisingly, these endothelial cells have mostly anaerobic metabolism, providing lactate to the adjacent hepatocytes.

High-resolution *in vivo* microscopy has revealed swelling and contracting of these cells as a response to vasoactive substances, suggesting that they participate in the regulation of blood flow [62]. A unique functional feature of the sinusoidal endothelial cells is their high endocytic capacity. This process is mediated by all sorts of scavenger receptors, providing the main pathway for clearance of weakened molecules from circulation [63, 64] (see Chapters 26–28). Endothelial sinusoidal cells also express several types of PRR (e.g. mannose receptor several TLRs) and are able to produce inflammatory cytokines (e.g. TNF and IL-6) as well as playing a significant role in the innate immunity. In spite of intensive studies, their role in adaptive immune reactions is still controversial [52].

The lifespan of sinusoidal endothelial cells is not known; they divide rarely and progenitor cells seem to be important in their maintenance. Two populations of progenitor cells can be distinguished [65]: the liver-resident population is thought to be responsible for normal cell turnover, while bone marrow-derived cells help to replenish the sinusoidal endothelial cells when necessary.

Hepatic immune cells

The human liver is exposed to 1.5 L of blood every minute and a massive load of harmless dietary and commensal antigens, to which it must remain tolerant. The predominantly tolerogenic role of the hepatic immune system is well known [52], but the liver is also exposed to a variety of viruses, bacteria, parasites, and metastatic tumor cells, so needs mechanisms to override immune tolerance. In addition, the liver's native immune system has a major regulatory role in the repair of the liver after cell injury and loss. The liver-centered immune system is largely segregated from the rest of the body's immune system [66, 67]. The human liver is estimated to contain about 10^{10} lymphocytes of different phenotypes, located along sinusoids and in portal tracts [66]. It includes a major fraction of the body's innate (native) immune capacity, as well as a small component of its acquired (adaptive) immune capacity [66, 67]. The major components of the hepatic immune system are innate lymphocytes, which include a variety of T cells and non-T cells that are able to respond rapidly to conserved ligands. These cells do not express T cell receptor (TCR) antigens. This group of immune cells includes NK cells, CD56$^+$ T cells, natural killer T (NKT) cells, γ/δ T cells, and mucosal associated invariant T (MAIT) cells. The liver contains multiple types of antigen

presenting cells such as hepatic myeloid dendritic cells (DCs), plasmacytoid DCs, $CD11^+$ DCs, and $NK1.1^+$ cytotoxic DCs [68]. In addition to the professional lymphocytes and DCs, several populations of hepatic resident cells (e.g. Kupffer cells, sinusoidal endothelial cells, stellate cells, and cholangiocytes) are also important and active players of the liver-centered immune system.

Macrophages

Macrophages are myeloid cells that are widely distributed throughout the tissues of mammalian organisms. The liver harbors 80% of all macrophages of the body. In addition, it is also patrolled by blood monocytes [69]. Hepatic macrophages can be divided into two classes based on their origin [70]: resident macrophages and bone marrow-derived macrophages. Resident macrophages of the liver, traditionally termed Kupffer cells, are established during embryonic development from the yolk sac and persist independent of blood monocytes. These cells self-renew during homeostatic conditions. Bone marrow-derived blood-borne monocytes give rise to monocyte-derived hepatic macrophages that are more characteristic of liver injury. Kupffer cells are not optimally suited to migration, so hepatic injuries massively recruit blood monocytes to the liver. These resemble Kupffer cells phenotypically, but they remain functionally different. Macrophages are highly versatile cells that play a substantial role in liver homeostasis, promoting and resolving inflammatory processes and fibrosis [69].

Liver macrophages are avidly phagocytic through C3 and Fc receptors, clearing the sinusoidal blood of relatively large particulate materials, including bacteria and weakened cells (worn-out erythrocytes, dead or damaged hepatocytes, etc.) [63, 64]. Together with sinusoidal endothelial cells they form the organism's major system for removing worn-out cells and proteins from perfusing blood. Activated macrophages produce many chemokines and cytokines that have a fundamental role in the implementation of the liver's acute-phase reaction, coordinating the responses of all the parenchymal cells to injury [67].

Myofibroblasts

Myofibroblasts are not present in the normal liver, but several cell types which are present physiologically can transform or be activated or transdifferentiated into the phenotype, which is the major source of extracellular matrix components and plays a basic role in pathological processes of the liver [71].

Hepatic stellate cells (HSCs) [72] are liver-resident mesenchymal cells which play an important role in liver physiology and pathology. They are found in the space of Disse, between the sinusoidal endothelial cells and hepatocytes. This special location makes them able to respond to numerous kinds of injury. In addition, by encircling the sinusoids they can function as pericytes and are thought to be the most important regulator of sinusoidal diameter and blood flow. They comprise about 1.5% of the parenchymal volume/mass [15] and are multifunctional (see Chapters 28 and 29). The embryonic origin of stellate cells is still uncertain. Most likely they originate from the septum transversum-derived mesothelial cells, but other options are still open. In the healthy liver they are the largest reservoir of vitamin A, hence their former name "fat-storing cells." When the liver is injured the stellate cells transdifferentiate into myofibroblasts and are the major producer of extracellular matrix (ECM). HSCs are important sources of cytokines and growth factors. This way they have impact on the proliferation, differentiation, and morphogenesis of the other hepatic cell types during liver development and regeneration [72].

Portal fibroblasts are spindle-shaped mesenchymal cells in the periportal connective tissue [73]. They are distinct from HSCs in both distribution and phenotype. They do not store vitamin A but express elastin and Thy-1. Portal fibroblasts take part in physiological ECM turnover and can contribute to the myofibroblast population in cholestatic liver injury.

Bone marrow-derived mesenchymal stem cells can also differentiate into myofibroblasts. The contribution of bone marrow cells is well established in the fibrotic processes of kidney and lung but it seems to be quite limited in the liver. Epithelial–mesenchymal transition (EMT) is another recently described mechanism. Both hepatocytes and cholangiocytes can undergo such phenotypic change in tissue culture, but lineage-tracing experiments in mice provide evidence against the role of EMT in myofibroblast generation in hepatic tissue *in vivo* [74, 75].

ONTOGENESIS OF THE LIVER

The evolutionary steps that resulted in the emergence of the mammalian liver with its multiple types of functioning cells are obscure. To the extent that the ontogenesis of the liver mirrors its phylogenesis, the embryonic development of the mammalian liver suggests the way in which the aggregation of multiple types of cell into the hepatic parenchyma may have evolved (for details see Chapter 2).

The development sequences of the liver in fish, birds, and mammals are similar [74–76], but endothelial cells do not appear to direct the emergence of endodermal cells from the gut during development of the liver in zebrafish [76]. Furthermore, endothelial cells with scavenger activity are in the gills and kidneys of cartilaginous and bony fish, and not in the liver as in all terrestrial animals [77]. The location of scavenger endothelial cells in the liver reflects a late step in the evolution of the mammalian liver. Nevertheless, the general pattern of expression of transcription factors and genes involved in liver development is conserved in all these species [78], suggesting a common transcriptional strategy for assembling the liver. Information on when this strategy first emerged awaits further genetic analysis of gut appendages in chordate ancestors of vertebrates.

Liver parenchymal repair

Three distinct processes have evolved to generate new hepatocytes needed to meet both increased physiological functional demands and to replace hepatocytes lost to trauma and/or toxicity. These processes comprise either a temporary reactivation of cell cycle transit in fully differentiated, mitotically quiescent hepatocytes, or the generation of entirely new hepatocyte lineages from adult liver stem cells (see Chapters 36 and 38).

The most direct and rapid parenchymal augmentation/replacement processes involve the induction of hepatocyte replication in the absence of a preceding increase in hepatocyte death, often associated with increased hepatic functional demand due to physiological need [78, 79]. Hyperplasia of hepatocytes by this mechanism enlarges the parenchymal mass and increases hepatocyte functional capacity. This process is regulated by the binding of ligands to hepatocyte nuclear receptors, nearly 50 of which have been identified [79, 80]. Nuclear receptors are transcription factors that, when bound to ligands, directly upregulate the combination of genes required to drive hepatocytes through the cell cycle [79, 80]. Several ligands for nuclear receptors (termed "primary hepatocyte mitogens"), including adrenal corticoids, bile acids, sex steroids, thyroid hormone, peroxisome proliferators, and 9-*cis*,*cis*-retinoic acid, directly stimulate the proliferation of hepatocytes and increase liver mass after binding to nuclear receptors [80]. Although it would appear that endothelial cells would be needed to support the additional hepatocytes, no documentation of coordinate endothelial cell proliferation has been presented; it is, however, possible that new endothelial cells could be derived from the bone marrow.

Next in process complexity and speed of response is the replacement of the diverse liver parenchyma by the sequential proliferation of all of the component cells (hepatocytes, cholangiocytes, endothelial cells, macrophages, stellate cells, and immunocytes), and the merging of the new cells into a tissue that closely resembles the functional units of the undamaged liver [78]. This process, which can replace up to 70% of the parenchymal mass in mammals, is often called "liver regeneration," although this is a misnomer since in mammals the part of the liver removed surgically does not "regenerate" in the way that body parts in certain lower animal species do. Instead, the liver, after resection, is enlarged by the expansion of remaining units (lobes), in a biological process defined as "compensatory hyperplasia." In contrast to liver repair in mammals, liver repair after partial hepatectomy in fish most intensively involves cells at the resection margin [81, 82] and may culminate in the regrowth (regeneration) of the resected tissue [81].

The cell proliferation phase of this reparative process in mammals has been subjected to intensive kinetic and regulatory analyses (see Chapter 45 and references therein). After tissue loss, residual hepatocytes are activated to proliferate within few hours. Hepatocyte proliferation begins at the portal ends of plates [83], and successive waves of hepatocyte proliferation ultimately involve virtually all residual hepatocytes [83]. Hepatocyte replacement is followed sequentially by proliferation of sinusoidal endothelial cells and macrophages [83, 84], and the other cells of the parenchymal matrix. To the extent that it has been elucidated (see Chapter 45), regulation of hepatocyte proliferation is regulated by a complex mixture of cytokines and growth factors [85]. Most of the regulatory molecules are produced by various liver cells or are released from storage sites within the liver [85], and many are components of the acute-phase reaction [86] and other elements of the liver's native immune system [87–89]. The less completely analyzed remodeling phase primarily involves endothelial cells and likely the other cells of the liver parenchyma. For example, proliferating hepatocytes initially form focal multicellular clumps [90, 91], which are cleaved into one-hepatocyte-wide plates by signaling from and separation by endothelial and stellate cells [90, 91]. Eventually, the remaining lobes increase exclusively by the enlargement of preexistent hepatic lobules, contrary to the physiological liver growth in young animals, when new lobules are formed [92].

Although known regulatory mechanisms drive the reparative process, the mechanism that "triggers" the onset of repair after loss of liver tissue is still obscure. Since the liver vasculature must accept the entire portal blood volume, it has long been suspected that the trigger may be the massive increase in portal blood flow per unit of residual mass that follows loss of liver tissue [93]. Increased portal blood flow and pressure cause shear stress in sinusoids [94], which produces a burst of nitric oxide and prostaglandin production by sinusoidal endothelial cells, possibly providing the molecular trigger [95, 96]. Alternatively (or in concert), early activation of the nuclear receptor mechanism of hepatocyte proliferation may function as a trigger [96], and it is possible that multiple alterations in the physiological status of the liver remaining after tissue loss may converge to produce a "mass action" trigger.

If the hepatocytes are compromised, there are alternative mechanisms of liver regeneration. Enlargement or hypertrophy of hepatocytes can compensate for the lost parenchyma [97], but this response usually provides just a transient solution.

There has been much debate about the participation of stem or progenitor cells in liver regeneration. A peculiar cell population, named after the shape of their nuclei as "oval cells," were observed in hepatocarcinogenesis experiments in rodents. Similar cells have been described in several other species and their emergence has been named "ductular reaction." There is convincing evidence that these cells can replace the lost liver parenchyma [98] and behave as the amplification compartment of hepatic stem cells. Several candidates for hepatic stem cells are known, but most data indicate that the terminal segment of the biliary system, the canals of Hering, harbor the adult hepatic stem cells.

Lineage-tracing experiments in rats [54, 55, 57, 99, 100] and zebrafish [101] demonstrated the regeneration of hepatocytes from biliary stem cells. The application of the cre/lox lineage tracing in mice did not support this prevailing model, because no biliary cell-derived hepatocytes were observed, although the hepatocytic origins of biliary cells and cholangiocarcinomas were demonstrated [102]. However, eventually hepatic progenitor cells of biliary origin with liver repopulation capacity were shown in mice following complete blockage of hepatocyte proliferation [103]. At present there seem to be general agreement [104] that both hepatocytes and cholangiocytes (or their subpopulations) are able to behave as stem cells, and under specific conditions are capable of regenerating both epithelial cell compartments of the liver. The capacity of these highly differentiated cells is also referred to as "plasticity" [105] but this is mostly a debate about terminology – how we should refer to a peculiar biological reaction. Initial observations indicate that these "back-up" stem cells, which support regenerative processes in rat and human, are organized along the branches of the portal vein [106, 107] and are regulated by elements of hepatic immunomodulation centered on the acute-phase reaction [98, 108], similar to the organization of liver architecture during embryonic development.

Although the subject of intensive scrutiny recently, there is no substantial evidence that hematopoietic stem cells or mesenchymal components of the liver are a significant source for the generation of hepatocytes or biliary epithelial cells in either humans or experimental animals [47]. This situation contrasts with the replenishment from hematopoietic sources of other cells of the liver parenchyma [47].

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