Leading article – Hepatology series

Cell biology of liver endothelial and Kupffer cells

Endothelial cells

Liver sinusoidal endothelial cells (LSEC) form a continuous, but fenestrated lining of the hepatic sinusoids.¹ Figure 1 shows rat liver endothelial lining as seen by scanning electron microscopy. The fenestrae are grouped in sieve plates. In rat liver, the fenestrae have an average diameter of about 150 nm in the centrilobular areas of the liver and of 175 nm in the periportal areas when measured in plastic embedded ultrathin sections in transmission electron microscopy. The shrinkage of the tissue due to the sample preparation is shown in results of scanning microscopy, with the diameters of fenestrae 105 to 110 nm.²

Fenestrae form open connections between the lumen of the sinusoid and the space of Disse (Fig 2).¹ It is assumed that the transport and exchange of fluid, solutes, and particles between the sinusoidal lumen and the space of Disse containing the parenchymal cell surface occur through these open fenestrae.^{2–4} This transport can be influenced by modulation of the diameter of the fenestrae by a variety of agents such as cytochalasin B,⁵ dimethyl nitrosamine,⁶ thioacetamide,⁷ ethyl alcohol,⁸ pantethine,⁹ nicotine,¹⁰ and extracellular matrix.¹¹ These agents change the diameter or number of fenestrae, or both. Data obtained by fluorescence microscopy show that the cytoskeleton of LSEC plays an important part in the modulation of fenestrae.^{12–13} Little information is available about mechanisms that regulate the diameter and number of fenestrae.

The fenestrated endothelial lining inhibits the passage of chylomicrons larger than 200–250 nm. Chylomicrons up to the size of fenestrae are present in the space of Disse, although larger chylomicrons are present in the blood, suggesting a filtration effect.^{4–14} Chylomicrons lose a

substantial amount of their triglycerides during circulation, resulting in decreasing diameters and comparative enrichment in cholesterol and cholesterol esters. The cholesterol rich remnants have access to the space of Disse through the fenestrae. Sieving of chylomicrons may play an important part in atherosclerosis and bile secretion.¹⁵ There also exists a reverse pathway for the transport of lipids, in the form of very low density lipoproteins. The Golgi apparatus of parenchymal cells transports vesicles with endogenously formed very low density lipoproteins to the cell surface. These vesicles are secreted into the space of Disse. Very low density lipoproteins particles have a diameter up to 90 nm, which allows them to pass through the endothelial filter.

It is known that rabbits have smaller fenestrae than rats. Smaller fenestrae cause longer circulation of cholesterol rich remnants before they are taken up by parenchymal cells. This finding may explain why rabbits are more sensitive to atherosclerosis than rats.¹⁶ Additionally, pantethine (a hypolipidemic drug), increases the size of fenestrae, at the same time lowering the cholesterol level in rabbits fed a cholesterol rich diet.⁹ Species specific differences of sinusoidal endothelial porosity were also found in chicken. Fraser *et al*¹⁷ showed that the hepatic sinusoidal endothelium of chicken ($2.2\% \pm 0.6$) is less porous than that of rats (12.0 ± 2.1) and rabbits (4.0 ± 1.5).

Wisse *et al*² showed the interaction between blood cells and the fenestrated sinusoidal wall using in vivo microscopy. Soft, fast moving erythrocytes passing the narrow sinusoids will promote the entrance of fluid, solid phase droplets or particles such as chylomicrons into the space of Disse by forced sieving. When rigid white blood cells pass through narrow sinusoids they compress the space of Disse, resulting in displacement of fluid (endothelial massage).

Figure 1: Scanning electron micrograph of the sinusoidal endothelium, showing clustering of fenestrae in sieve plates (arrows). Numerous villi, derived from the parenchymal cells (PC) can be seen in the space of Disse (SD). Bar: 1 μ m.

Figure 2: Transmission electron micrograph of an endothelial cell. In the cell processes, fenestrae (F) are apparent. SD: space of Disse; P=parenchymal cell. Bar: 1 μ m.

Defenestration and capillarisation of sinusoids plays an important part in diseases such as hepatic fibrosis. It was shown in humans, that chronic alcohol consumption leads to almost defenestrated sinusoids.¹⁸ At the same time, deposition of extracellular matrix in the space of Disse occurs.¹⁹ Rogers *et al*⁶ found in the dimethyl nitrosamine rat model of cirrhosis a rapid reduction in size and number of fenestrae before the onset of cirrhosis.

CYTOCHEMICAL MARKERS

To discriminate sinusoidal from vascular endothelial cells, or from other liver cells (Kupffer, fat storing, pit, parenchymal, and bile duct epithelial cells), ultrastructural, enzyme cytochemical, immunocytochemical, and in situ receptor ligand binding studies can be used.

Enzyme cytochemistry

Kupffer cells are normally the only sinusoidal cells that stain positively with endogenous peroxidase. Using mild fixation procedures, however, rat LSEC may also give a positive reaction with endogenous peroxidase.²⁰ This contrasts with earlier findings where no staining was found, even without fixation of the liver.²¹ In addition it has been reported that mouse LSEC also stain positively with this enzyme, even after normal fixation protocols.²² These findings show that this enzyme marker can be used with caution to distinguish between Kupffer cells and LSEC in rat.

Immunocytochemical markers

Although the successful production of LSEC specific monoclonal antibodies has been reported, these antibodies do in fact label other types of microvascular endothelia as well.²³ Positive staining with antiserum to von Willebrand factor (of factor VIII related antigen) is commonly used as a cytochemical criterion to prove the presence of LSEC. For two reasons this marker should be used with caution. Firstly, the presence of von Willebrand factor is normally used as a marker of endothelium in general,²⁴ that is, provided this marker is present in LSEC, it cannot disclose whether the cells are of sinusoidal or vascular origin. Secondly, the mere presence of von Willebrand factor antigen in LSEC is still a disputed issue. While some authors use this marker without mentioning the controversy,^{25 26} other reports claim that LSEC are devoid of, or stain only weakly for this antigen.^{27 28} It is interesting to note that a controversy also exists regarding the presence of factor VIII procoagulant antigen (FVIII:C) in LSEC. Whereas some authors have reported the presence of activity or antigen, or both of FVIII:C in rat and human LSEC and not in other liver cells,^{29 30} other researchers claim that the parenchymal cells contain more of this procoagulant antigen than LSEC.^{31 32} Until these controversies have been settled, markers other than von Willebrand factor and FVIII:C should be preferred whenever there is a need to discriminate LSEC from other cell types.

FUNCTIONAL MARKERS

An important physiological function of LSEC is to free the bloodstream from a variety of macromolecular waste products. To this end the cells are geared to receptor mediated endocytosis and carry high affinity receptors for both foreign and physiological substances (see later). Many of these substances are endocytosed almost exclusively and with a remarkable efficiency by LSEC.

This feature has been used to vitally stain these cells. Smedsrød *et al*³³ conjugated fluorescein isothiocyanate to hyaluronan,³⁴ chondroitin sulphate proteoglycan,³⁵ collagen alpha chains,³³ and N-terminal propeptide of procollagen type I.³⁶ They showed that only LSEC accumulate the dye. Staining is equally efficient and cell specific when the conjugates are given intravenously or when supplemented to cells in culture. Therefore, in systems of viable cells this way of distinguishing LSEC from other types of cells is probably the most reliable and specific method. It should be noted that uptake of low density lipoprotein, artificially modified by acetylation, and conjugated with the fluorochrome 1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanine perchlorate (DiI-AcLDL) has also been suggested as a specific marker of liver endothelial cells. In fact, it has been shown that AcLDL given intravenously labelled with either DiI or ¹²⁵I is cleared almost exclusively by LSEC.^{37 38} As this substances is also used as a probe of the macrophage scavenger receptor,³⁹ and as many authors are now using it routinely as a marker of many different non-LSEC types of endothelia in culture,^{40 41} the use of DiI-AcLDL as a LSEC specific marker seems adequate only after intravenous injection of the probe.

ENDOCYTOSIS

Sinusoidal endothelial cells have a high endocytic capacity (vide supra). This function of endothelial cells is reflected morphologically by the presence of numerous endocytic vesicles.⁴² After methods had been worked out to prepare cultures of pure LSEC,⁴³⁻⁴⁵ it soon became clear that the cells could take up an array of different substances by receptor mediated endocytosis.⁴⁶ These molecules included unphysiologically modified serum proteins, such as acetylated low density lipoprotein and formaldehyde treated serum albumin. Today we know that this feature of LSEC reflects their important role as a scavenger system that clears the blood from many different macromolecular waste products, which originate from normal turnover processes in different tissues. The first clue to this scavenger system of physiological waste molecules was obtained from studies on the turnover of the connective tissue polysaccharide hyaluronan.³⁴ At the time when those studies were undertaken (late 1970s), hardly anybody would question the notion that the hepatic reticuloendothelial system was made up exclusively of Kupffer cells. Therefore, the finding that circulating hyaluronan was sequestered almost exclusively by LSEC came as a surprise, assigning a novel physiological role to these cells. During the years that followed this finding, a number of additional connective tissue macromolecules and other substances have been found to be cleared mainly by LSEC (Table). Based on these findings it was logical to include LSEC functionally to the hepatic reticuloendothelial system.⁴⁶ A share of the work obviously exists between the two hepatic reticuloendothelial system members, Kupffer

Some physiological macromolecular ligands that are cleared from the circulation by receptor mediated endocytosis almost exclusively in liver endothelial cells

Ligand	Receptor	Reference
Hyaluronan	Hyaluronan receptor	34
Chondroitin sulphate	Hyaluronan receptor	35
Collagen α chains	Collagen α chain receptor	33
N-terminal propeptides of procollagen types I and II	Scavenger receptor	36
C-terminal propeptide of procollagen type I	Mannose receptor	162
Laminin	Unknown	163
Nidogen	Unknown	163

cells removing particles by phagocytosis, and LSEC scavenging soluble macromolecules and small particles largely by receptor mediated endocytosis. It is interesting to note that receptors that have traditionally been viewed as typical for macrophages – that is, the mannose receptor and scavenger receptor – are in fact carried by LSEC. Intravenous injection of macromolecules that are ligands for these receptors are taken up either mainly by LSEC (AcLDL^{37,47} and mannose terminated ligands⁴⁸) or jointly by LSEC and Kupffer cells (oxidised low density lipoprotein⁴⁹). Because procedures elaborated to establish pure monolayer cultures of functionally intact LSEC are comparatively novel, the processes of receptor mediated endocytosis, intracellular transport, and degradation of macromolecules have only recently been investigated in these cells.

Studies on endocytosis by the mannose receptor in LSEC have shown an extremely rapid surface receptor half life ($t_{1/2} = \pm 10$ seconds⁵⁰). Electron and fluorescence microscopic studies on intracellular transport of ligands that were endocytosed by the mannose receptor (gold conjugated ovalbumin⁵¹), the hyaluronan receptor (gold conjugated chondroitin sulphate proteoglycan⁵²), the collagen α chain receptor (fluorescein labelled denatured type I collagen⁵³), and the scavenger receptor (fluorescein labelled *N*-terminal propeptide of type I procollagen³⁶) all show the same morphological pattern: shortly after binding to coated regions of the plasma membrane, and internalisation, the ligand becomes located in small (± 0.1 μm diameter) vesicles in the periphery of the cell. After about five minutes the ligand is seen in larger vesicles, which contain the probe (gold particles or fluorescein) along the inner aspect of the vesicle. These vesicles, appearing as rings, grow in diameter, and after about 20 minutes attain rather large sizes (1 μm or more), the probe still being located at the periphery. At later time points the ligand is seen perinuclearly in vesicles whose diameter is constantly being reduced. After 60 minutes the diameter is about 0.1 μm , and the probe now seems to be equally distributed throughout the lumen of the vesicles. Degradation of the ligand starts at about the time of occurrence of the largest 'ring structures' (which is after about 20 minutes). Despite a comparatively efficient degradation during the first hour of incubation, it takes several hours to achieve complete degradation of endocytosed collagen α chains and *N*-terminal propeptide of type I procollagen. The finding that degradation of endocytosed ¹²⁵I-labelled ligands give free ¹²⁵I⁻ as the main degradation product, shows that LSEC contain a dehalogenase.³⁶ When incubation is carried out at 20°C or in the presence of monensin, a carboxylic ionophore that abolishes the proton pump, the intracellular transport of endocytosed ligand is halted at the level of the 'ring structures'.⁵³ These treatments also totally abolish degradation. The exact nature of the different endocytic structures seen during intracellular transport is at present unclear, and it has been difficult to identify these structures as early or late endosomes, or lysosomes. Nevertheless, electron microscopic findings showed the same morphology of intracellular trafficking of endocytosed chondroitin sulphate proteoglycan labelled with colloidal gold.⁵² After 20–40 minutes the probe was associated with the inner aspects of large vacuoles. Thereafter, it was seen in the lumen of smaller perinuclearly located vesicles considered as lysosomes because of their content of acid phosphatase. Using immunohistochemistry at the electron microscopic level to mark out the lysosomal proteinase cathepsin D and lysosomal membrane glycoprotein 120, Stang *et al*⁵⁴ found that gold labelled mannose terminal tissue plasminogen activator appeared in early endosomes of LSEC one minute after

intravenous injection. After six and 12 minutes the probe was located in late endosomes and lysosomes, respectively. This finding shows that intracellular transport of endocytosed ligands in LSEC is much faster in the intact liver than in cultured cells. Misquith *et al*⁵⁵ showed that lysosomal degradation of formaldehyde treated albumin, a ligand for the LSEC scavenger receptor, is a two step process, entailing first a 'transfer' lysosome, and finally an 'accumulation' lysosome. Kindberg *et al*⁵⁶ similarly found that endocytosed ovalbumin was degraded in two populations of lysosomes. The degradation of hyaluronan has been studied in some detail (see ⁵⁷ for a review): intralysosomal degradation of this large connective tissue polysaccharide by the concerted action of hyaluronidase, β -D-glucuronidase and β -*N*-acetyl-D-hexosaminidase yields *N*-acetyl glucosamine and D-glucuronic acid, both of which are transported across the lysosomal membrane to the cytoplasm. Here, D-glucuronic acid enters the pentose phosphate and glycolytic pathways to yield carbon dioxide and lactate, whereas *N*-acetylglucosamine is phosphorylated to *N*-acetylglucosamine-6-phosphate, and deacetylated by a specific deacetylase, which is present in Kupffer cells and LSEC but not in parenchymal cells. After deacetylation, the aminosugar enters metabolic reactions yielding acetate, ammonia, and lactate. These products are secreted from LSEC and taken up by parenchymal cells.⁵⁸ Using aerobic metabolism, parenchymal cells turn the metabolites into water, carbon dioxide, and urea. The final degradation products are excreted into the sinusoidal circulation and leave the liver through the hepatic vein.

PROSTANOID PRODUCTION BY LIVER SINUSOIDAL ENDOTHELIAL CELLS

The prostanoids are oxygenated derivatives of C₂₀ fatty acids, mainly arachidonic acid, generated by the cyclo-oxygenase pathway. There are two important classes of prostanoids: the prostaglandins and the thromboxanes. Prostanoids are not stored. They are synthesised *de novo* and are released in response to a stimulus.⁵⁹ Vascular endothelial cells release prostanoids, especially prostacyclin, when properly stimulated by agents such as histamine or thrombin. Prostacyclin was first detected in the vascular wall by Moncada *et al* in 1977.⁶⁰ Since then its biological activity as a vasodilator and a potent inhibitor of platelet activation has been described. As LSEC differ both morphologically, for example, fenestration clustered in sieve plates and functionally, for example, expression of Fc receptors,⁶¹ from endothelial cells of other sources, data concerning prostanoids release by vascular endothelial cells from large vessels cannot be readily applied to the liver.

Cultured endothelial cells from guinea pig liver produce both 6-keto-prostaglandin F_{1 α} , the stable degradation product of prostacyclin, significant amounts of thromboxane B₂, and small amounts of prostaglandin E₂, upon incubation with [¹⁴C]arachidonic acid.⁶² In contrast, short term cultured rat endothelial cells produce only low amounts of 6-keto-prostaglandin F_{1 α} and more thromboxane B₂, prostaglandin E₂, and prostaglandin D₂ when incubated with arachidonic acid.⁶³

It is known that endotoxin from the gut,^{64,65} and cytokines produced by Kupffer cells such as interleukin 1⁶⁶ or tumour necrosis factor,⁶⁷ may influence liver function by prostanoids produced (6-keto-prostaglandin F_{1 α} , prostaglandin E₂, and thromboxane B₂) by LSEC.²⁵

Prostanoids, especially prostaglandin E₂ and prostaglandin D₂, have profound effects on hepatocyte metabolism and on haemodynamics. However, not only short term haemodynamic and metabolic consequences of

prostanoids release have to be considered. It is well known that prostaglandins, especially of the E type modulate immune functions through interleukin 1 production.⁶⁸ Prostaglandins of the E type seem to attenuate damage to the liver by toxic agents such as CCl₄,⁶⁹ galactosamine,⁷⁰ ethanol,⁷⁰ bromobenzene,⁷¹ aflatoxin,⁷² acetaminophen,⁷⁰ and cyclosporin.⁷³ Prostacyclin has also been reported to prevent damage from CCl₄,⁷⁴ or hypoxia.⁷⁵ The mechanism of protection of hepatocytes by prostaglandins is poorly understood. Recently it has been shown in human patients that the administration of prostaglandin E₂ analogues can prevent the fatal outcome of fulminant viral hepatitis.⁷⁶ Prostanoids, especially prostacyclin and prostaglandin E₂, produced by endotoxin stimulated endothelial cells are obviously not able to protect hepatocytes from the endotoxin induced fulminant hepatitis in animals, sensitised by D-galactosamine. Probably the toxic effect of mediators, such as tumour necrosis factor and leukotrienes,⁷⁷ produced by neutrophils adhering to endothelial cells under the influence of tumour necrosis factor,⁷⁸ overwhelm the endogenous defence mechanism of endothelial cells and hepatocytes in the presence of D-galactosamine.

Nevertheless, although endothelial cells proved to be refractory to tumour necrosis factor as far as prostanoids metabolism is concerned, other immune mediators, for example, interleukin 1 may act on endothelial cells, and their prostanoids release could in turn influence inflammatory processes⁷⁹ or fibroblast proliferation.⁸⁰

INTERACTION WITH BLOOD CELLS DURING INFLAMMATION

The entry of circulating leucocytes into acute and chronic inflammatory lesions is commonly referred to as recruitment.⁸¹ This leucocyte recruitment from the blood compartment is a crucial determinant for the induction and expression of immunity and inflammation. At the start of this process, soluble mediators generated in response to tissue injury increase the adhesion of leucocytes to the regional microvasculature and augment migration of attached cells into tissues.⁸² Leucocyte extravasation entails adhesion to endothelial cells and response to tissue derived chemotactic signals. This adhesion is a complex phenomenon requiring the mutual recognition and interaction of multiple adhesive receptors, which are expressed both on leucocytes and endothelial cells. Endothelial cells play an active part in the control of leucocyte recruitment by producing cytokines that activate leucocytes, and by expressing membrane proteins that are a substratum for adhesion of circulating cells.^{83–87} There are three important families of leucocyte adhesion molecules: the Ig related molecules, the integrins, and the selectins (homing receptors).⁸⁸ The Ig related molecules include CD2 (sheep erythrocyte receptor) and its ligand CD58 (LFA-3), neural adhesion molecule CD56 (NCAM) and vascular adhesion molecule VCAM-1, CD54 (intracellular adhesion molecule, ICAM-1), ICAM-2, and ICAM-3 (binding to integrin).^{89–93} The expression of these adhesion molecules is regulated by bacterial products (for example, lipopolysaccharide) and cytokines.^{83–87} Different leucocyte populations recognise 'activated' endothelial cells by means of different receptors. Polymorphonuclear leucocytes recognise ELAM-1, whereas lymphocytes bind to VCAM-1. Monocytes also recognise VCAM-1 in addition to ELAM-1. Natural killer cells adhere through the CD18/CD11 pathway in addition to the α_4/β_1 VCAM-1 pathway.

In lipopolysaccharide induced liver inflammation, an increased number of neutrophils has been reported as early as 30 minutes to one hour after an intravenous lipopolysaccharide challenge.^{78 94} Tumour necrosis factor has been

found to mediate this phenomenon.⁷⁸ Lipopolysaccharide induces Kupffer cells to secrete tumour necrosis factor. This last cytokine causes neutrophils to adhere to sinusoidal endothelial cells.^{95 96}

INTERACTION WITH TUMOUR CELLS

Tumour metastasis is the primary element responsible for the morbidity and mortality of malignant cancers. Clinical and experimental findings show that many malignant tumour cells preferentially metastasise to distant organs, and that secondary tumour formation can lead to eventual death of the host.^{97–101} Historically, two explanations have been proposed for this organ specific metastasis. The first one is that tumour cells are trapped mechanically in the first capillary bed encountered during blood borne transit.¹⁰² The second explanation assumes specific interaction of the tumour cells with the microenvironment of a particular organ.^{103–108} The adhesion of malignant cells to microvascular endothelial cells at specific organ sites seems to participate in determining distribution and organ preference of certain metastases.^{97 100 101 109} Furthermore, the arrest and metastasis of circulating cells can be promoted in locally inflamed areas. This effect may result from the local release of cytokines that activate the endothelial cell adhesion receptors such as VCAM-1 or E-selectin.¹¹⁰

The liver is a key target organ in development of haematogenous metastases of primary cancers with venous drainage into the portal system.¹¹¹ The liver is the most common site of metastasis of colon cancer.¹¹² Liver metastasising murine colon carcinoma cells were seen to invade the liver from portal tributaries and to be present in subcapsular areas, or as intraparenchymal microfoci. Electron microscopic findings further showed the presence of these tumour cells in hepatic sinusoids in apposition to the endothelial and parenchymal cells.¹¹³ Other metastatic tumour cells (lymphosarcoma and mammary adenocarcinoma) were also shown to interact with sinusoidal endothelial cells, both in vivo and in vitro.^{107 114} Specific cell surface molecules on endothelial cells participate in the adherence of metastatic cells. Highly metastatic cells adhere strongly whereas low metastatic cells do not adhere onto cultured endothelial cells.¹⁰⁷ The subsequent step of crossing the endothelial barrier of the liver sinusoids seems to be crucial in determining the success of establishing metastatic foci. By doing so, the tumour cell will probably escape the hepatic natural defence system, consisting of Kupffer cells and pit cells.¹¹⁵

SYNTHESIS OF EXTRACELLULAR MATRIX PROTEINS

Liver endothelial cells contribute to extracellular matrix protein deposition in the space of Disse. In vitro, sinusoidal endothelial cells produce collagen type IV,¹¹⁶ and fibronectin.¹¹⁷ Northern hybridisation analysis of freshly isolated and purified endothelial cells from normal rat liver showed the presence of collagen $\alpha 1$ (IV) transcripts. No evidence for fibronectin transcripts was found.¹¹⁸ Two papers report endothelial cells to contain collagen $\alpha 1$ (III) mRNA.^{119 120} Clement *et al* reported immunoreactivity for collagen type III in these cells.^{121 122}

SECRETION OF PEPTIDE MEDIATORS

Interleukin 1 α

Interleukin 1 α is one of the major cytokines participating in the regulation of many immunological and inflammatory responses. In the liver interleukin 1 α is produced by

both endothelial¹²³ and Kupffer cells.¹²⁴ Recent data¹²⁵ show that interleukin 1 α does not only elicit adhering of leucocytes in sinusoids, but also activates the phagocytic function of the endothelium.

Interleukin 6

Endotoxin stimulates interleukin 6 activity in cultured endothelial cells.²⁵ In addition, culture supernatants of stimulated endothelial cells evoke an acute phase reaction by cultured hepatocytes.²⁵

Kupffer cells

The ultrastructural characteristics of Kupffer cells are well characterised by electron microscopy (Fig 3).^{21 126} Kupffer cells are found in the sinusoidal lumen on top of or between endothelial cells and sometimes also in the space of Disse. They have an irregular shape with many cytoplasmic extensions and contain a large number of lysosomes and phagosomes, associated with their endocytic function. In addition, they contain a well developed endoplasmic reticulum, Golgi complex, and secretory vesicles. Kupffer cells can be obtained from the livers of various animals through perfusion with proteolytic enzymes (typically a collagenase/pronase mixture), followed by density gradient centrifugation and centrifugal elutriation of the resulting cell suspension.¹²⁷ Bouwens *et al*¹²⁸ found that under normal conditions, Kupffer cells have a long turnover time in the liver. Experimental inflammatory conditions, however, induce a strong hyperplasia of the Kupffer cell population. The same authors also showed that in certain instances, Kupffer cells can be recruited from bone marrow progenitors, but resident Kupffer cells can also proliferate in the liver.¹²⁹ After partial hepatectomy, the Kupffer cell population is regenerated by local proliferation.¹³⁰ Using morphological, functional, and enzyme cytochemical characteristics, it can be shown that besides resident Kupffer cells, also macrophages, derived from recruited monocytes, occur in the liver sinusoids, during hepatic inflammation.¹³¹

Figure 3: Transmission electron micrograph of a Kupffer cell. The microvilli intermingle with those of the parenchymal cell (arrow). The cytoplasm contains a large number of dense bodies varying in shape, density, and diameter. L=lumen of the sinusoid; E=erythrocyte; SD=space of Disse; P=parenchymal cell; N=nucleus. Bar: 1 mm.

FUNCTIONS

Endocytosis

The position of Kupffer cells within the liver sinusoid makes these cells the first macrophages to come into contact with gut derived foreign and potentially noxious material such as viruses, bacteria, protozoa, and their products. The endocytotic functions of Kupffer cells have been extensively described.^{115 132}

Secretion of peptide mediators

Tumour necrosis factor α – Tumour necrosis factor α is a pleiotropic cytokine of 17 kDa, derived from monocytes and activated T cells.¹³³ When stimulated in vitro with lipopolysaccharide, Kupffer cells synthesise tumour necrosis factor α mRNA and secrete the protein in the culture medium.^{134 135} Kupffer cells were for a long time thought to be the only source of tumour necrosis factor α in the liver, but in situ hybridisation showed the presence of tumour necrosis factor α mRNA not only in individual Kupffer cells but also in bile duct epithelium.¹³⁶

Interleukin 1 α – Interleukin 1 α is one of the major cytokines participating in the regulation of many immunological and inflammatory responses. In the liver interleukin 1 α is produced by both endothelial cells¹²³ and Kupffer cells.¹²⁴ Recent data¹²⁵ show that interleukin 1 α not only elicits adhering of leucocytes in sinusoids, but also activates phagocytic function of the sinusoidal endothelium.

Interleukin 6 – Interleukin 6 is the major mediator for induction of acute phase proteins.¹³⁷ Kupffer cells in primary culture synthesise interleukin 6. Synthesis can be stimulated by tumour necrosis factor α and interleukin 1.¹³⁸

Transforming growth factor β – Transforming growth factor β is a gene superfamily of growth factors with multiple effects on different target cells. Transforming growth factor β_1 is currently the best characterised cytokine of this family. This multipotent protein is ubiquitously present in both normal tissues and transformed cells,¹³⁹ and almost all cells have receptors for it.¹⁴⁰ It is secreted in inactive form and local factors – that is, lowered pH and proteases may activate the molecule.¹³⁹ Besides transforming growth factor β_1 four other isoforms are known, transforming growth factor β_{2-5} . Transforming growth factor β_{1-3} are found in mammals and chickens. Transforming growth factor $\beta_{4,5}$ are present only in chickens and amphibians (for a recent review see ¹⁴¹).

Transforming growth factor β is present in conditioned Kupffer cells medium and stimulates collagen synthesis by cultured fat storing cells,¹⁴² while inhibiting proliferation of these cells.^{143–145} It has also an inhibitory effect on the collagen synthesis of parenchymal cells.¹⁴⁶

In freshly isolated cells from normal rat liver, northern hybridisation analysis showed the presence of transforming growth factor β_1 mRNA in Kupffer cells and to a lesser extent in fat storing cells. No message is found in hepatocytes or endothelial cells.¹⁴⁷

Secretion of prostanoids and oxygen species

Kupffer cells play an important part in the immune system through phagocytosis and killing of invading micro-organisms. The contact between a suitable particle and its receptor on the surface of the Kupffer cell, elicits the production of compounds that include arachidonate metabolites (prostanoids) and reactive oxygen species (superoxide and nitric oxide ¹⁴⁸). A number of particulate

and soluble agents, including phorbol ester, zymosan, arachidonic acid, and the calcium ionophore A23187 are known to stimulate in Kupffer cells the formation of prostanoids, mainly prostaglandins prostaglandin D₂, prostaglandin E₂, and thromboxane.^{149 150} The release of prostaglandins is mainly controlled by a phospholipase A₂, which liberates arachidonic acid from membrane lipids. By two different pathways, one involving cyclo-oxygenase and the other lipo-oxygenase, both prostaglandins and leukotrienes are synthesised starting from arachidonate.

Zymosan and phorbol-12-myristate-13-acetate (PMA) elicit superoxide O₂⁻.¹⁴⁸ Superoxide formation is catalysed by NADPH oxidase residing in the plasma membrane. The superoxide produced by Kupffer cells helps the macrophage to inactivate and destroy phagocytosed organisms and particles. Lipopolysaccharide activated rat Kupffer cells can synthesise nitric oxide from L-arginine. An L-arginine dependent process in Kupffer cells leads to the inhibition of protein synthesis in cocultured hepatocytes.¹⁵¹ The mediator of this inhibition is probably nitric oxide. It has been shown that the stimulus induced release of prostanoids but not of superoxide depends on the extracellular concentrations of Na⁺. Na⁺/H⁺ exchange and concomitant changes in the cellular pH participate in the stimulus response coupling.¹⁵² Earlier findings showed that calcium ions may also participate in the stimulus induced production and release of prostanoids by Kupffer cells. It has been shown in cell free extracts of rat Kupffer cells that phospholipase A₂ was activated at free Ca²⁺ concentrations from 100 nM to 1 µM in the presence of 4 mM free Mg²⁺. Ca²⁺ alone increased phospholipase A₂ activity at a high Ca²⁺ concentration of 1 mM, whereas Mg²⁺ alone had only little stimulatory effect.¹⁵³ The stimulus induced prostaglandin E₂ formation from endogenous lipids is inhibited by dexamethasone pretreatment of cultured rat Kupffer cells. Dexamethasone has no influence when free arachidonic acid is used as a substrate. This shows that glucocorticoids inhibit the liberation of the unsaturated fatty acid from phospholipids and suggests hormone induced formation of a protein that modifies the activity of phospholipase A₂. Lipocortin is a likely candidate.¹⁴⁹

INTERACTIONS OF KUPFFER CELLS WITH TUMOUR CELLS

The cytotoxic activity of murine and rat Kupffer cells to various tumour cell lines in vitro has been reported by several authors.¹⁵⁴⁻¹⁵⁸ It has recently been shown that human Kupffer cells also have a tumoricidal action against colonic adenocarcinoma in vitro.¹⁵⁹ Generally, Kupffer cells require activation by immunomodulators such as muramyl peptides, lipopolysaccharides, lymphokines, and interferon γ. The in vivo role of Kupffer cells was further shown by animal studies where activation and suppression of Kupffer cells affect the number and size of liver metastases after intraportal challenge with tumour cells.¹⁶⁰ Treatment with inhibitors of Kupffer cell function such as silica, gadolinium salts or antimacrophage serum resulted in increased tumour growth whereas stimulators of Kupffer cell function such as zymosan, glucan, muramyl dipeptide, and *Corynebacterium parvum* decreased tumour growth in vivo. It was further shown that the in vitro cytotoxicity of Kupffer cells against colon carcinoma cells was gradually built up during the development of liver metastases.¹⁵⁸ These studies show that Kupffer cells may play a part in the destruction of tumour cells and that activation of these cells through the application of biological response modifiers may provide an attractive alternative

because properly activated macrophages are known to be cytotoxic against a large variety of tumour targets irrespective of specificity.^{155 161}

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This is the last paper in the Hepatology series of leading articles.

- 1 Wisse E. An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoids. *J Ultrastruct Res* 1970; 31: 125-50.
- 2 Wisse E, De Zanger RB, Charles K, Vandersmissen P, McCuskey RS. The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. *Hepatology* 1985; 5: 683-92.
- 3 Fraser R, Day WA, Fernando NS. Atherosclerosis and the liver sieve. In: Kirm IA, Knook DL, Wisse E, eds. *Cells of the hepatic sinusoid*. Rijswijk: Kupffer Cell Foundation, 1986: 317.
- 4 Naito M, Wisse E. Filtration effect of endothelial fenestrations on chylomicron transport in neonatal rat liver sinusoids. *Cell Tissue Res* 1978; 190: 371-82.
- 5 Steffan AM, Gendralt JL, Kirm A. Increase in the number of mouse fenestrae in mouse endothelial liver cells by altering the cytoskeleton with cytochalasin B. *Hepatology* 1987; 7: 1230-8.
- 6 Rogers GWT, Dobbs BR, Fraser R. Decreased hepatic uptake of cholesterol and retinol in the dimethyl nitrosamine rat model of liver cirrhosis. *Liver* 1992; 12: 326-9.
- 7 Mori T, Okanoue T, Sawa Y, Hori N, Ohta M, Kagawa K. Defenestration of the sinusoidal endothelial cell in a rat model of liver cirrhosis. *Hepatology* 1993; 17: 891-7.
- 8 Clark SA, Angus HB, Cook HB, Oxner RBG, George PM, Fraser R. Defenestration of hepatic sinusoids as a cause of hyperlipoproteinaemia in alcoholics. *Lancet* 1988; ii: 1225-7.
- 9 Fraser R, Clark SA, Bowler LM, Day WA. Panthetine inhibits diet-induced hypercholesterolaemia by dilating the liver sieve. *NZ Med J* 1988; 101: 86-7.
- 10 Fraser R, Clark SA, Day WA, Murray FEM. Nicotine decreases the porosity of the rat liver sieve: a possible mechanism for hypercholesterolaemia. *Br J Exp Pathol* 1988; 69: 345-50.
- 11 McGuire RF, Bissell DM, Boyles J, Roll FJ. Role of extracellular matrix in regulating fenestrations of sinusoidal endothelial cells isolated from normal rat liver. *Hepatology* 1992; 15: 989-97.
- 12 Arias IM. The biology of hepatic endothelial cell fenestrae. In: Schaffer F, Popper H, eds. *Progress in liver disease*. Philadelphia: WB Saunders, 1990: 11.
- 13 Van Der Smitten P, Van Bossuyt H, Charels K, Wisse E. The structure and the function of the cytoskeleton in sinusoidal endothelial cells in the rat liver. In: Kirm IA, Knook DL, Wisse E, eds. *Cells of the hepatic sinusoid*. Rijswijk: Kupffer Cell Foundation, 1986: 517.
- 14 De Zanger RB, Wisse E. The filtration effect of rat liver fenestrated sinusoidal endothelium on the passage of (remnant) chylomicrons to the space of Disse. In: Knook DL, Wisse E, eds. *Sinusoidal liver cells*. Amsterdam: Elsevier, 1982: 69.
- 15 Fraser R, Bosanquet AG, Day WA. Filtration of chylomicrons by the liver may influence cholesterol metabolism and atherosclerosis. *Atherosclerosis* 1978; 29: 113-23.
- 16 Wright PL, Smith KF, Day WA, Fraser R. Small liver fenestrae may explain the susceptibility of rabbits to atherosclerosis. *Atherosclerosis* 1983; 3: 344-8.
- 17 Fraser R, Heslop VR, Murray FEM, Day WA. Ultrastructural studies of the portal transport of fat in chickens. *Br J Exp Pathol* 1986; 67: 783-91.
- 18 Horn T, Christofferson P, Henriksen JH. Alcoholic liver injury: defenestration in non-cirrhotic livers - a scanning electron microscopic study. *Hepatology* 1987; 7: 77-82.
- 19 Horn T, Junge J, Christofferson P. Alcoholic liver injury: early changes of the Disse space in acinar zone 3. *Liver* 1985; 6: 301-10.
- 20 Litwin JA. Peroxidase-positive endothelial cells in rat liver. *Cell Tissue Res* 1984; 238: 635-42.
- 21 Wisse E. Observations on the fine structure and peroxidase chemistry of normal rat liver Kupffer cells. *J Ultrastruct Res* 1974; 46: 393-426.
- 22 Stohr G, Deinmann W, Fahimi HD. Peroxidase-positive endothelial cells. *J Histochem Cytochem* 1978; 26: 409-11.
- 23 Product catalogue of Monosan. Uden, The Netherlands, 83.
- 24 Jaffe EA, Nachman RL, Becker CG, Minick CR. Culture of human endothelial cells derived from umbilical veins. *J Clin Invest* 1973; 52: 2745-56.
- 25 Rieder H, Ramadori G, Allman KH, Meyer zum Buschenfelde KH. Prostanoids release of cultured liver sinusoidal endothelial cells in response to endotoxin and tumor necrosis factor. Comparison with umbilical vein endothelial cells. *J Hepatol* 1990; 11: 359-66.
- 26 Harrison RL, Boudreau R. Human hepatic sinusoidal endothelial cells in culture produce von Willebrand factor and contain Weibel-Palade bodies. *Liver* 1989; 9: 242-9.
- 27 Terada T, Nakanuma Y. Expression of ABH blood group antigens, Ulex europaeus agglutinin I, and type IV collagen in the sinusoids of hepatocellular carcinoma. *Arch Pathol Lab Med* 1991; 115: 50-5.
- 28 Petrovic L, Burroughs A, Scheuer PJ. Hepatic sinusoidal endothelium: Ulex lectin binding. *Histopathology* 1989; 14: 233-43.
- 29 Hellman L, Smedsrød B, Sandberg H, Pettersson U. Secretion of a coagulant factor VIII activity and antigen by in vitro cultivated rat liver sinusoidal endothelial cells. *Br J Haematol* 1989; 73: 348-55.
- 30 Stel HV, Van Der Kwast TH, Veerman ECI. Detection of factor VIII/coagulant antigen in human liver tissue. *Nature* 1983; 303: 530-2.
- 31 Zelechowska MG, Van Mourik JA, Brodiewicz-Proba T. Ultrastructural localization of factor VIII procoagulant antigen in human liver hepatocytes. *Nature* 1985; 317: 729-30.
- 32 Kelly DA, Summerfield JA, Tuddenham EGD. Localization of factor VIII:C antigen in guinea pig tissues and isolated liver cell fractions. *Br J Haematol* 1984; 56: 535-43.
- 33 Smedsrød B, Johanssen S, Pertoft H. Studies in vivo and vitro on the uptake and degradation of soluble collagen alpha(I) chains in rat liver endothelial and Kupffer cells. *Biochem J* 1985; 228: 415-24.
- 34 Smedsrød B, Pertoft H, Eriksson S, Fraser JRE, Laurent TC. Studies in vitro on the uptake and degradation of sodium hyaluronate in rat liver endothelial cells. *Biochem J* 1984; 223: 617-26.
- 35 Smedsrød B, Kjellen L, Pertoft H. Endocytosis and degradation of chondroitin sulphate by liver endothelial cells. *Biochem J* 1985; 229: 63-71.
- 36 Melkko J, Hellevik T, Risteli L, Risteli J, Smedsrød B. Clearance of aminoterminal propeptides of types I and III procollagen is a physiological function of the scavenger receptor in liver endothelial cells. *J Exp Med* 1994; 179: 405-12.
- 37 Nagelkerke JF, Barto KP, Berkel TJC. In vivo and in vitro uptake and degradation of acetylated low density lipoproteins in vivo is mediated by specific endothelial cells. *J Biol Chem* 1983; 258: 12221-7.

- 38 Pitas RE, Boyles J, Mahley RW, Bissell MD. Uptake of chemically modified low density lipoproteins in vivo is mediated by specific endothelial cells. *J Cell Biol* 1985; 100: 103-17.
- 39 Brown MS, Goldstein JL. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Ann Rev Biochem* 1983; 52: 223-61.
- 40 Stein O, Stein Y. Bovine aortic endothelial cells display macrophage-like properties towards acetylated [125I]-labelled low density lipoprotein. *Biochim Biophys Acta* 1980; 620: 631-5.
- 41 Voyta JC, Via DP, Butterfield CE, Zetter BR. Identification and isolation of endothelial cells based on their increased uptake of acetylated-low density lipoprotein. *J Cell Biol* 1984; 99: 2030-40.
- 42 Wisse E. An ultrastructural characterization of the endothelial cell in rat liver sinusoids under normal and various experimental conditions, as a contribution to the distinction between endothelial and Kupffer cells. *J Ultrastruct Res* 1972; 38: 528-62.
- 43 Knook DL, Sleyster E. Separation of Kupffer and endothelial cells of the rat liver by centrifugal elutriation. *Exp Cell Res* 1976; 99: 444-9.
- 44 Smedsrød B, Pertofti H. Preparation of pure hepatocytes and reticuloendothelial cells in high yield from a single rat liver by means of Percoll centrifugation and selective adherence. *J Leukoc Biol* 1985; 38: 213-30.
- 45 Braet F, De Zanger RB, Sasaoki T, Bakeland M, Janssens P, Smedsrød B, et al. Assessment of a method of isolation, purification and cultivation of rat liver sinusoidal endothelial cells. *Lab Invest* 1994; 70: 944-52.
- 46 Smedsrød B, Pertofti H, Gustafson S, Laurent TC. Scavenger functions of the liver endothelial cell. *Biochem J* 1990; 266: 313-27.
- 47 Blomhoff R, Drevon CA, Eskild W, Helgerud P, Norum KR, Berg T. Clearance of acetyl low density lipoprotein by rat liver endothelial cells. Implications for hepatic cholesterol metabolism. *J Biol Chem* 1984; 259: 8898-903.
- 48 Kindberg G, Magnusson S, Berg T, Smedsrød B. Receptor-mediated endocytosis of ovalbumin by two carbohydrate-specific receptors in rat liver cells. The intracellular transport of ovalbumin to lysosomes is faster in liver endothelial cells than in parenchymal cells. *Biochem J* 1990; 257: 197-203.
- 49 Van Berkel TJ, De Rijke YB, Kruijt JK. Different fate in vivo of oxidatively modified low density lipoprotein and acetylated low density lipoprotein in rats. *J Biol Chem* 1991; 266: 2282-9.
- 50 Magnusson S, Berg T. Extremely rapid endocytosis mediated by the mannose receptor of sinusoidal rat liver endothelial cells. *Biochem J* 1989; 257: 651-6.
- 51 Stang E, Kindberg G, Berg T, Roos N. Endocytosis mediated by the mannose receptor in liver endothelial cells. An immunocytochemical study. *Eur J Cell Biol* 1990; 52: 67-76.
- 52 Smedsrød B, Malmgren M, Ericsson J, Laurent TC. Morphological studies on endocytosis of chondroitin sulphate proteoglycan by rat liver endothelial cells. *Cell Tissue Res* 1988; 253: 39-45.
- 53 Hellevik T, Bondevik A, Smedsrød B. Intracellular trafficking of endocytosed collagen in rat liver endothelial cells. In: Knook DL, Wisse E, eds. *Cells of the hepatic sinusoid*. Leiden: Kupffer Cell Foundation, 1993: 431-3.
- 54 Stang E, Krause J, Kindberg G, Roos N. Hepatic endocytosis of tissue-type plasminogen activator with special reference to the sinusoidal cells. In: Wisse E, Knook DL, McCuskey, eds. *Cells of the hepatic sinusoid*. Leiden: The Kupffer Cell Foundation, 1991: 542-5.
- 55 Misquith S, Wattiaux-De Coninck S, Wattiaux R. Uptake and intracellular transport in rat liver of formaldehyde-treated bovine serum albumin labelled with 125I-tyramine cellobiose. *Eur J Biochem* 1988; 174: 691-7.
- 56 Kindberg G, Stang E, Andersen KJ, Roos N, Berg T. Intracellular transport of endocytosed proteins in rat liver endothelial cells. *Biochem J* 1990; 270: 205-11.
- 57 Smedsrød B. Cellular events in the uptake and degradation of hyaluronan. *Adv Drug Deliv Rev* 1991; 7: 265-78.
- 58 David H, Kasner G, Krause W, Behrisch D, Wenzel K. Ultrastructure and quantitative composition of isolated endothelial cells of rat liver. *Exp Pathol* 1990; 39: 95-101.
- 59 Coleman RA, Kennedy I, Humphrey PPA, Bruce K, Lumley P. In: Emmett JC, ed. *Comprehensive medicinal chemistry*. Oxford: Pergamon, 1990: 643-714.
- 60 Moncada S, Higgs EA, Vane JR. Human arterial and venous tissues generate prostacyclin (prostaglandin X) a potent inhibitor of platelet aggregation. *Lancet* 1977; i: 18-21.
- 61 Van Der Laan-Klamer SM, Harms G, Hardonk MJ. *Histochemistry* 1986; 84: 257-62.
- 62 Shaw RG, Johnson AR, Schulz WW, Zahlten RN, Combes R. Sinusoidal endothelial cells from normal guinea pig liver, isolation, culture and characterization. *Hepatology* 1984; 4: 591-602.
- 63 Eyhorn S, Schlöyer HJ, Henninger HP, Dieter P, Hermann R, Woort-Menken M, et al. Rat hepatic sinusoidal endothelial cells in monolayer culture. Biochemical and ultrastructural characteristics. *J Hepatol* 1988; 6: 23-35.
- 64 Prytz H, Holst-Christensen J, Korner B, Liehr H. Portal venous and systemic endotoxaemia in patients without liver disease and systemic endotoxaemia in patients with liver cirrhosis. *Scand J Gastroenterol* 1976; 11: 857-63.
- 65 Jacob AJ, Goldberg PK, Bloom N, Degenshein GA, Kozinn PJ. Endotoxin and bacteria in portal blood. *Gastroenterology* 1977; 72: 1268-70.
- 66 Leser HG, Debatin KM, Northof H, Gerns D. Kupffer-cell-enriched non-parenchymal liver cells and peritoneal macrophages differ in prostaglandin E release, production of interleukin 1 and tumor cytotoxicity. In: Knook DL, Wisse E, eds. *Sinusoidal liver cells*. Amsterdam: Elsevier Biomedical Press, 1982: 369-76.
- 67 Karck U, Peters T, Decker K. The release of tumor necrosis factor by LPS-treated rat Kupffer cells and its regulation. In Wisse E, Knook DL, Decker K, eds. *Cells of hepatic sinusoid*. Leiden: Kupffer Cell Foundation, 1989: 210-1.
- 68 Kunkel SL, Chensue SW, Phan SH. Prostaglandins as endogenous mediators of interleukin 1 production. *J Immunol* 1986; 136: 186-92.
- 69 Caratxolo A, Magri V, Stuzzolo R, Famulari C, Durange V. 16,16-Dimethyl prostaglandin E1 prevents the changes of diazepam metabolism induced by carbo tetrachloride in the rat. *IRCS Pharmacol* 1985; 13: 551-2.
- 70 Stachura J, Tarnawski A, Ivey KJ, Ruwart MJ, Rush BD, Friedle NM, et al. 16,16-Dimethyl prostaglandin E2 protection of rat liver against acute injury by galactosamine, acetaminophen, ethanol and ANIT. *Gastroenterology* 1981; 80: 1349.
- 71 Funek-Brentano C, Tinel M, Degott C, Letterou P, Babany G, Pessayre D. Protective effect of 16,16-dimethyl prostaglandin E2 on the hepatocyte of bromobenzene in mice. *Biochem Pharmacol* 1984; 33: 89-94.
- 72 Rush BD, Wilkinson KF, Nichols NM, Ochoa R, Brunden MN, Ruwart MJ. Hepatic protection by 16,16-dimethyl prostaglandin E2 against acute aflatoxin B1-induced injury in the rat. *Prostaglandins* 1989; 97: 683-93.
- 73 Makowka L, Gilas T, Falk R, Lopantini W, Phillips MJ, Falk J. Prevention of chronic cyclosporin (Cs)-induced hepatotoxicity by 16,16-dimethyl prostaglandin E2 (dmPGE2). *Gastroenterology* 1985; 88: 1676.
- 74 Lapis K, Jeney A, Divald A, Vajta G, Zalantai A, Schaff Z. Experimental studies on the effect of hepatoprotective compounds. *Tokai J Exp Clin Med (suppl)* 1986; 11: 135-45.
- 75 Sikujara O, Monden M, Toyoshima K, Okamura J, Kosaki G. Cytoprotective effect of prostaglandin I2 on ischemia-induced hepatic cell injury. *Transplantation* 1983; 36: 238-43.
- 76 Sinclair SB, Greig PD, Blendis LM. Biomedical and clinical response of fulminant hepatitis to administration of prostaglandin E. *J Clin Invest* 1989; 84: 1063-9.
- 77 Keppler D, Huber M, Baument T. Leukotrienes as mediators in diseases of the liver. *Semin Liver Dis* 1988; 8: 357-66.
- 78 Schlöyer HJ, Laaff H, Peters T, Woort-Menken M, Estler HC, Karck U, et al. Involvement of tumor necrosis factor in endotoxin-triggered neutrophil adherence to sinusoidal endothelial cells of mouse liver and its modulation in acute phase. *J Hepatol* 1988; 7: 239-49.
- 79 Tate GA, Mandell BG, Schumacher HR, Zurier RB. Suppression of acute inflammation by 15-methyl-prostaglandin E1. *Lab Invest* 1988; 59: 192-9.
- 80 Libby P, Warner SJC, Friedman GB. Interleukin 1: a mitogen for human vascular smooth muscle that induce the release of growth-inhibitory prostanooids. *J Clin Invest* 1988; 81: 487-98.
- 81 Stoolman LM, Kaldjian E. Adhesion molecules involved in the trafficking of normal and malignant leukocytes. *Invasion Metastasis* 1992; 12: 101-11.
- 82 Butcher EC. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell* 1991; 67: 1033-6.
- 83 Larson RS, Springer TA. Structure and function of leukocyte integrins. *Immunol Rev* 1990; 114: 181-217.
- 84 Carlos TM, Harlan JM. Membrane proteins involved in phagocyte adherence to endothelium. *Immunol Rev* 1990; 114: 5-28.
- 85 Dransfield I, Buckle AM, Hogg N. Early events of the immune response mediated by leukocyte integrins. *Immunol Rev* 1990; 114: 29-44.
- 86 Patayorro M, Prieto J, Rincon T, Timonen C, Lundberg C, Lindbom L, et al. Leukocyte-cell adhesion: a molecular process fundamental in leukocyte physiology. *Immunol Rev* 1989; 114: 1303.
- 87 Mantovani A, Dejana E. Cytokines as communication signals between leukocytes and endothelial cells. *Immunol Today* 1989; 10: 370-5.
- 88 Moller G. Lymphocyte homing. *Immunol Rev* 1989; 108: 5-161.
- 89 Marlin SD, Springer TA. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). *Cells* 1987; 51: 813.
- 90 Staunton DE, Dustin ML, Springer TA. Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. *Nature (Lond)* 1989; 339: 61-4.
- 91 Bevilacqua MP, Stengelin S, Gimbrone MA, Seed B. Endothelial leukocyte molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science* 1989; 24: 1160.
- 92 Rice GE, Bevilacqua MP. An inducible endothelial surface glycoprotein mediates melanoma adhesion. *Science* 1989; 246: 1303-6.
- 93 Osborn LC, Hession R, Tizzard C, Vassallo S, Luhnowsky S, Chi-Rosso S, et al. Direct expression cloning of vascular cell adhesion protein 1, a cytokine-induced endothelial protein that binds to lymphocytes. *Cell* 1989; 59: 1203-11.
- 94 Onda M, Tobia M, Andoh T, Shiota A. Ultrastructural studies of experimental endotoxin shock in the liver and spleen: therapeutic effects of low-dose heparin on reticuloendothelial disturbances. *Circ Shock* 1986; 18: 11-9.
- 95 Mannel DN, Meltzer MS, Mergenhagen SE. Generation and characterization of a lipopolysaccharide-induced and serum-derived cytotoxic factor for tumor cells. *Infect Immun* 1980; 28: 204-11.
- 96 Schlöyer HJ, Karck V, Ganter U, Hermann R, Decker K. Enhancement of neutrophil adherence to isolated rat liver sinusoidal endothelial cells by supernatants of lipopolysaccharide-activated monocytes. Role of tumor necrosis factor. *J Hepatol* 1987; 5: 311-21.
- 97 Nicholson GL, Poste G. Tumor cell diversity and host responses in cancer metastases - part 1 - properties of metastatic cells. *Curr Probl Cancer* 1982; 7: 1-83.
- 98 Naito S, Von Eschenbach AC, Fidler IJ. Different growth pattern and biological behavior of human renal cell carcinoma implanted into different organs of nude mice. *J Natl Cancer Inst* 1987; 78: 377-85.
- 99 Murphy GP. Management of metastatic prostatic cancer. *Prog Clin Biol Res* 1988; 277: 127-34.
- 100 Nicholson GL. Organ specificity of tumor metastasis: role of preferential adhesion invasion and growth of malignant cells at specific secondary sites. *Cancer Metastasis Rev* 1988; 7: 143-88.
- 101 Auerbach R, Lu W, Pardon E, Gumkowski F, Kaminski G, Kaminski M. Specificity of adhesion between murine tumor cells and capillary endothelium: an in vitro correlate of preferential metastasis in vivo. *Cancer Res* 1987; 47: 1492-6.
- 102 Ewing J. A treatise on tumors. *Neoplastic disease*. Philadelphia: Saunders, 1982.
- 103 Weiss L. Random and nonrandom processes in metastasis, and metastasis efficiency. *Invasion Metastasis* 1983; 3: 193-208.
- 104 Irimura T, Nicolson GL. Carbohydrate chain analysis by lectin binding to mixtures of glycoproteins separated by polyacrylamide slab-gel electrophoresis with in situ chemical modification. *Carbohydrate Res* 1984; 115: 209-20.
- 105 Hart IR. The role of animal models in the study of experimental metastasis. In: Liotta L, Hart IR, eds. *Tumor invasion and metastasis*. The Hague: Martinus Nijhoff, 1982: 1-14.
- 106 Hart IR, Fidler IJ. The role of organ selectivity in the determination of metastases of B16 melanoma. *Cancer Res* 1980; 40: 2281-7.
- 107 Roos E, Middlekoop OP, Van De Pavert IV. Adhesion of tumor cells to hepatocytes: different mechanisms of mammary carcinoma compared with lymphosarcoma cells. *J Natl Cancer Inst* 1984; 73: 963-9.
- 108 Schirmacher V. Cancer metastasis: experimental approaches, theoretical concepts, and impacts for treatment strategies. *Adv Cancer Res* 1985; 43: 831-73.
- 109 Tressler RJ, Belloni PN, Nicolson GL. Correlation of inhibition of adhesion of large cell lymphoma and hepatic sinusoidal cells by RGD-containing peptide polymers with metastatic potential: role of integrin-dependent and -independent adhesion mechanism. *Cancer Comm* 1989; 1: 55-63.
- 110 Abelda SM. Biology of disease. Role of integrins and other cell adhesion molecules in tumor progression and metastasis. *Lab Invest* 1993; 68: 4-17.
- 111 Barbera-Guillen E, Weiss L. Cancer-cell traffic in the liver. III. Lethal deformation of B16 melanoma cells in liver sinusoids. *Int J Cancer* 1993; 54: 880-4.
- 112 Panis Y, Nordlinger B, Deleor R, Herve JP, Infante J, Kuhnle M, et al. Experimental colorectal metastases. Influence of sex, immunological status and liver regeneration. *J Hepatol* 1990; 11: 53-7.
- 113 Bresalier RS, Hujanen ES, Raper SE, Roll FJ, Itzkowitz SH, Martin GR, et al. An animal model for colon cancer metastasis: establishment and characterization of murine cell lines with enhanced liver-metastasizing ability. *Cancer Res* 1987; 47: 1398-406.
- 114 Roos E, Dingemans KP. Phagocytosis of tumor cells by Kupffer cells in vivo and in the perfused mouse liver. In: Wisse E, Knook DL, eds. *Kupffer and other liver sinusoidal cells*. Amsterdam: Elsevier Biomedical Press, 1977: 183-90.
- 115 Bouwens L, Geerts A, Van Bossuyt H, Wisse E. Recent insights into the function of hepatic sinusoidal cells. *Neth J Med* 1987; 31: 129-48.
- 116 Irving MG, Roll RJ, Huang S. Characterization and culture of sinusoidal endothelium from normal rat liver: lipoprotein uptake and collagen phenotype. *Gastroenterology* 1984; 87: 1233-47.
- 117 Rieder H, Ramadori G, Dienes HP. Sinusoidal endothelial cells from guinea pig liver synthesize and secrete fibronectin in vitro. *Hepatology* 1987; 7: 856-64.
- 118 Geerts A, Greenwel P, Cunningham M, De Bleser P, Rogiers V, Wisse E, et al. Identification of connective tissue gene transcripts in freshly isolated parenchymal, endothelial, Kupffer and fat-storing cells by Northern hybridization analysis. *J Hepatol* 1993; 19: 148-58.
- 119 Maher J, McGuire R. Extracellular matrix gene expression increases preferentially in rat lipocytes and sinusoidal endothelial cells during hepatic fibrosis in vivo. *J Clin Invest* 1990; 86: 1641-8.
- 120 Weiner F, Shah A, Biempica L, Zern M, Czaja M. The effects of hepatic fibrosis on Ito cell gene expression. *Matrix* 1992; 12: 36-43.
- 121 Clement B, Emonard H, Rissel M. Cellular origin of collagen and fibronectin in the liver. *Cell Mol Biol* 1984; 30: 489-96.
- 122 Clement B, Grimaud J, Campion J, Deugneiz Y, Guillozo A. Cell types involved in collagen and fibronectin production in normal and fibrotic human liver. *Hepatology* 1986; 6: 225-34.
- 123 Feder LS, McCloskey TW, Laskin DL. Characterization of interleukin-1 (IL-1) and interleukin-6 (IL-6) production by resident and lipopolysaccharide (LPS) activated hepatic macrophages and endothelial cells. In: Wisse E, Knook DL, McCuskey RS, eds. *Cells of the hepatic sinusoid*. Leiden: Kupffer Cell Foundation, 1991: 37-9.
- 124 Bauer J, Birmelin M, Northoff GH, Northemann W, Tran-Thai TA, Ueberer H, et al. Induction of alpha 2-macroglobulin in vivo and hepatic primary cultures: synergistic action of glucocorticoids and a Kupffer cell-derived factor. *FEBS Lett* 1984; 177: 89-94.
- 125 Nishida J, McDonnell D, Krasovich MA, Ekataksin W, McCuskey RS. Effects of interleukin-1α on hepatic phagocytic cells and sinusoidal microcirculation. In: Knook DL, Wisse E, eds. *Cells of the hepatic sinusoid*. Leiden: Kupffer Cell Foundation, 1993: 148-51.
- 126 Wisse E. Kupffer cell reactions in rat liver under various conditions as observed under the electron microscope. *J Ultrastruct Res* 1974; 46: 499-520.
- 127 Van Bossuyt H, Bouwens L, Wisse E. Isolation, purification and culture of sinusoidal liver cells. In: Bioulac-Sage P, Balabaud C, eds. *Sinusoids in human liver: health and disease*. Rijswijk: Kupffer Cell Foundation, 1988: 1-16.

- 128 Bouwens L, Baekeland M, De Zanger RB, Wisse E. Quantitation, tissue distribution and proliferation kinetics of Kupffer cells in normal rat liver. *Hepatology* 1986; **6**: 718–22.
- 129 Bouwens L, Knook D, Wisse E. Local proliferation and extrahepatic recruitment of liver macrophages (Kupffer cells) in partial-body irradiated rats. *J Leukoc Biol* 1986; **39**: 687–97.
- 130 Bouwens L, Baekeland M, Eisse E. Importance of local proliferation in the expending Kupffer cell population of rat liver after zymosan stimulation and partial hepatectomy. *Hepatology* 1984; **4**: 213–9.
- 131 Bouwens L, Wisse E. Proliferation, kinetics and fate of monocytes in rat during a zymosan-induced inflammation. *J Leukoc Biol* 1985; **37**: 531–43.
- 132 Wake K, Decker K, Kim A, Knook DL, McCuskey RS, Bouwens L, et al. Cell biology and kinetics of Kupffer cells in the liver. *Int Rev Cytol* 1989; **118**: 173–229.
- 133 Beutler B, Cerami A. The biology of cachectin/TNF – a primary mediator of host response. *Ann Rev Immunol* 1989; **7**: 625–55.
- 134 Freudenberg MA, Galanos C. Bacterial lipopolysaccharides: structures, metabolism and mechanisms of action. *Int Rev Immunol* 1990; **6**: 207–21.
- 135 Karcz U, Peters T, Decker K. The release of tumor necrosis factor from endotoxin stimulated rat Kupffer cells is regulated by prostaglandin E2 and dexamethasone. *J Hepatol* 1988; **27**: 352–61.
- 136 Hoffmann R, Grewe M, Estler H, Schulze-Specking A, Decker K. Detection of different TNF- α -mRNA-synthesizing cells in the LPS perfused rat liver by non-radioactive in situ hybridization. In: Knook DL, Wisse E, eds. *Cells of the hepatic sinusoid*. Leiden: Kupffer Cell Foundation, 1993: 17–20.
- 137 Andus T, Geiger T, Hirano T, Kishimoto T, Tran-Thi TA, Decker K, et al. Regulation of synthesis and secretion of major rat acute-phase proteins by recombinant human interleukin-6 (BSF-2, IL-6) in hepatocyte primary cultures. *Eur J Biochem* 1988; **173**: 287–93.
- 138 Busam KJ, Bauer TM, Bauer J, Gerok W, Decker K. Interleukin-6 release by rat liver macrophages. *J Hepatol* 1990; **11**: 367–73.
- 139 Sporn MB, Roberts AB, Wakefield L, De Crombrughe M. Some recent advances in the chemistry and biology of transforming growth factor- β . *J Cell Biol* 1987; **105**: 1039–45.
- 140 Wakefield LM, Smith DM, Masui T, Harris CC, Sporn MB. Distribution and modulation of the cellular receptor for transforming growth factor- β . *J Cell Biol* 1987; **105**: 965–75.
- 141 Massague J. The transforming growth factor- β family. *Ann Rev Cell Biol* 1990; **6**: 597–641.
- 142 Matsuoka M, Tsukamoto H. Stimulation of hepatic lipocyte production by Kupffer cell-derived transforming growth factor β : implication for a pathogenetic role in alcoholic liver fibrogenesis. *Hepatology* 1990; **11**: 599–605.
- 143 Davis BH. Transforming growth factor- β responsiveness is modulated by the extracellular collagen matrix during hepatic Ito cell culture. *J Cell Physiol* 1988; **136**: 547–53.
- 144 Matsuoka M, Pham N, Tsukamoto H. Different effects of interleukin-1 α , tumor necrosis factor α , and transforming growth factor- β 1 on cell proliferation and collagen formation by cultured fat-storing cells. *Liver* 1989; **9**: 71–8.
- 145 Czaja MJ, Weiner FR, Flanders KC, Giambrone M, Wind R, Biempica L, et al. In vitro and in vivo association of transforming growth factor- β 1 with hepatic fibrosis. *J Cell Biol* 1989; **108**: 2477–82.
- 146 Tsutsumi Y, Kakumu S, Yoshioka K, Aroa M, Inoue M, Wakita T. Effects of various cytokines on collagen synthesis by normal rat hepatocytes in primary cultures and fibroblasts. *Digestion* 1989; **44**: 191–9.
- 147 De Bleser P, Geerts A, Lazou J, Niki T, Seynaeve C, Rogiers V, et al. Distribution of TGF- β _{1,2,3} transcripts in cells of normal and CCl₄ treated rat liver. In: Knook DL, Wisse E, eds. *Cells of the hepatic sinusoid*. Leiden: Kupffer Cell Foundation, 1993: 214–7.
- 148 Decker K. Biologically active products of stimulated liver macrophages (Kupffer cells). *Eur J Biochem* 1990; **192**: 245–61.
- 149 Dieter P, Schulze-Specking A, Decker K. Differential inhibition of prostaglandin and superoxide production by dexamethasone in primary cultured rat Kupffer cells. *Eur J Biochem* 1986; **159**: 451–7.
- 150 Dieter P, Schulze-Specking A, Decker K. Ca²⁺ requirement of prostanoid but not of superoxide production by rat Kupffer cells. *Eur J Biochem* 1988; **177**: 61–7.
- 151 Billiar TR, Curran RD, Stuehr DJ, West MA, Beutis BG, Simmons RL. An L-arginine-dependent mechanism mediates Kupffer cell inhibition of hepatocyte protein synthesis in vitro. *J Exp Med* 1989; **169**: 1467–72.
- 152 Dieter P, Schulze-Specking A, Karcz V, Decker K. Prostaglandin release but not superoxide production by rat Kupffer cells stimulated in vitro depends on Na⁺/H⁺ exchange. *Eur J Biochem* 1987; **170**: 201–6.
- 153 Krause H, Dieter P, Schulze-Specking A, Ballhorn A, Ferber E, Decker K. Synergetic effect of magnesium and calcium ions in the activation of phospholipase A₂ of liver macrophages. *Biochem Biophys Res Comm* 1991; **175**: 532–6.
- 154 Decker T, Kiderlen AF, Lohmann-Matthes ML. Liver macrophages (Kupffer cells) as cytotoxic effectors in extracellular and intracellular cytotoxicity. *Infect Immun* 1985; **50**: 358–64.
- 155 Daemen T, Veninga A, Roerdink FM, Scherphof GL. In vitro activation of rat liver macrophages to tumoricidal activity by free or liposome-encapsulated muramyl dipeptide. *Cancer Res* 1986; **46**: 4330–5.
- 156 Cohen SA, Salazar D, Von Muenchhausen W, Werner-Wasik M, Nolan JP. Natural anti-tumor defense system of murine liver. *J Leukoc Biol* 1985; **37**: 559–69.
- 157 Malter M, Friedrich E, Suss R. Liver as a tumor cell killing organ: Kupffer cells and natural killers. *Cancer Res* 1986; **46**: 3055–60.
- 158 Thomas C, Nijenhuis AM, Daemen T, Scherphof GL. Tumoricidal and kinetic response of rat Kupffer cells to challenge with syngeneic colon adenocarcinoma cells or liposomal immunomodulators. In: Knook DL, Wisse E, eds. *Cells of the hepatic sinusoid*. Leiden: Kupffer Cell Foundation, 1993: 496–9.
- 159 Roh MS, Wang L, Oyedele C, LeRoux ME, Curley SA, Pollock RE, et al. Human Kupffer cells are cytotoxic against human colon adenocarcinoma. *Surgery* 1990; **108**: 400–4.
- 160 Phillips NC. Kupffer cells and liver metastasis. *Cancer Metastasis Rev* 1989; **8**: 231–52.
- 161 Fidler IJ, Schroit AJ. Recognition and destruction of neoplastic cells by activated macrophages or altered self. *Biochim Biophys Acta* 1988; **948**: 151–73.
- 162 Smedsrod B, Melkko J, Risteli L, Risteli J. Circulating carboxyterminal propeptide of procollagen type I is cleared mainly via the mannose receptor in liver endothelial cells. *Biochem J* 1990; **271**: 345–50.
- 163 Smedsrod B, Paulsson M, Johansson S. Uptake and degradation in vivo and in vitro of laminin and nidogen by rat liver cells. *Biochem J* 1986; **261**: 37–42.