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. 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 periporal 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). 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,6 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.1213 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.416 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.13 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 al17 showed that the hepatic sinusoidal endothelium of chicken (2.2%±0.6) is less porous than that of rats (12.0±2.1) and rabbits (4.0±1.5).

Wisse et al2 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).
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. At the same time, deposition of extracellular matrix in the space of Disse occurs. Rogers et al found in the dimethyl nitroamine 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, of liver, Kupffer cells (Kupffer, fat storing pit, parenchymal, and bile duct epithelial cells), ultrastructural, enzyme cytochemical, immunoctyochemical, 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. This contrasts with earlier findings where no staining was found, even without fixation of the liver. In addition it has been reported that mouse LSEC also stain positively with this enzyme, even after normal fixation protocols. 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. 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, 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, other reports claim that LSEC are devoid of, or stain only weakly for this antigen. 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, other researchers claim that the parenchymal cells contain more of this procoagulant antigen than LSEC. 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. Smedsroed et al conjugated fluorescein isothiocyanate to hyaluronan, collagen alpha chains, and N-terminal propeptide of procollagen type I. 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 1,2-dioctadecyl-3,3',3'-tetramethyl-indocarbocyanine perchlorate (DiIC14) has also been suggested as a specific marker of liver endothelial cells. In fact, it has been shown that DiIC14 given intravenously labelled with either DiI or DiI is cleared almost exclusively by LSEC. As this substance is also used as a probe of the macropage scavenger receptor, and as many authors are now using it routinely as a marker of many different non-LSEC types of endothelia in culture, the use of DiIC14 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. After methods had been worked out to prepare cultures of pure LSEC, it soon became clear that the cells could take up an array of different substances by receptor mediated endocytosis. 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. At the time when these 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. Based on these findings it was logical to include LSEC functionally to the hepatic reticuloendothelial system. A share of the work obviously exists between the two hepatic reticuloendothelial system members, Kupffer and...
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 (Ac-LDL \(^{37, 47}\) and mannose terminated ligands \(^{48}\)) or jointly by LSEC and Kupffer cells (oxidised low density lipoprotein \(^{49}\)). 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 \((t_{1/2} = \pm 10 \text{ seconds})^{50}\). Electron and fluorescence microscopic studies on intracellular transport of ligands that were endocytosed by the mannose receptor (gold conjugated ovalbumin \(^{51}\)), the hyaluronan receptor (gold conjugated chondroitin sulphate proteoglycan \(^{52}\)), the collagen \(\alpha\) chain receptor (fluorescein labelled denatured type I collagen \(^{53}\)), and the scavenger receptor (fluorescein labelled \(N\)-terminal propeptide of type I procollagen \(^{49}\)) all show the same morphological pattern: shortly after binding to coated regions of the plasma membrane, and internalisation, the ligand becomes located in small \((\pm 0.1 \mu \text{m}\) 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 vessel. These vesicles, appearing as rings, grow in diameter, and after about 20 minutes attain rather large sizes \((1 \mu \text{m} \text{ or more})\), the probe still being located at the periphery. At later times the ligand is seen perinuclearly in vesicles whose diameter is constantly being reduced. After 60 minutes the diameter is about 0.1 \mu m, 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 \(\alpha\) chains and \(N\)-terminal propeptide of type I procollagen. The finding that degradation of endocytosed \(^{125}\)I-labelled ligands give free \(^{125}\)I as the major degradation product, shows that LSEC contain a dehalogenase. \(^{56}\) 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): intralyosomal degradation of this large connective tissue polysaccharide by the concerted action of hyaluronidase, \(\beta\)-D-glucuronidase and \(\beta\)-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 enter 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 \(C_{20}\) fatty acids, mainly arachidonic acid, generated by the cyclooxygenase 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 \(F_{c}\) 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 \(F_{1\alpha}\) the stable degradation product of prostacyclin, significant amounts of thromboxane \(B_{2}\) and small amounts of prostaglandin \(E_{2}\), upon incubation with \(^{14}\)C-arachidonic acid. \(^{62}\) In contrast, short term cultured rat endothelial cells produce only low amounts of 6-keto-prostaglandin \(F_{1\alpha}\), and more thromboxane \(B_{2}\), prostaglandin \(E_{2}\) and prostaglandin \(D_{2}\) when incubated with arachidonic acid. \(^{63}\)

It is known that endotoxin from the gut, \(^{64}\) and cytokines produced by Kupffer cells such as interleukin 1 \(^{66}\) or tumour necrosis factor, \(^{67}\) may influence liver function by prostanoids produced (6-keto-prostaglandin \(F_{1\alpha}\), prostaglandin \(E_{2}\) and thromboxane \(B_{2}\)) by LSEC. \(^{25}\)

Prostanoids, especially prostaglandin \(E_{2}\) and prostaglandin \(D_{2}\), 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. Prostaglandins of the E type seem to attenuate damage to the liver by toxic agents such as CCl₄, galactosamine, ethanol, bromobenzene, aflatoxin, acetonaphthen, and cyclosporin. Prostaglandin E₂, produced by endotoxin stimulated endothelial cells are obviously not able to protect hepatocytes form the endotoxin induced fulminant hepatitis in animals, sensitised by D-galactosamine. Probably the toxic effect of mediators, such as tumour necrosis factor and leukotrienes, produced by neutrophils adhering to endothelial cells under the influence of tumour necrosis factor, 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, cytokines like interleukin 1 may act on endothelial cells, and their prostanoids release could in turn influence inflammatory processes or fibroblast proliferation.

INTERACTION WITH BLOOD CELLS DURING INFLAMMATION

The entry of circulating leucocytes into acute and chronic inflammatory lesions is commonly referred to as recruitment. 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. Leucocyte extravasation 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. There are three important families of leucocyte adhesion molecules: the Ig related molecules, the integrins, and the selectins (homing receptors). 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). The expression of these adhesion molecules is regulated by bacterial products (for example, lipopolysaccharide) and cytokines. 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 αβ, 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. Tumour necrosis factor has been found to mediate this phenomenon. Lipopolysaccharide induces Kupffer cells to secrete tumour necrosis factor. This last cytokine causes neutrophils to adhere to sinusoidal endothelial cells.

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. 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. The second explanation assumes specific interaction of the tumour cells with the microenvironment of a particular organ. 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. 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.

The liver is a key target organ in development of haematogenous metastases of primary cancers with venous drainage into the portal system. The liver is the most common site of metastasis of colon cancer. 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. Other metastatic tumour cells (lymphosarcoma and mammary adenocarcinoma) were also shown to interact with sinusoidal endothelial cells, both in vivo and in vitro. Specific cellsurface 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. 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.

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, and fibronectin. Northern hybridisation analysis of freshly isolated and purified endothelial cells from normal rat liver showed the presence of collagen a1(IV) transcripts. No evidence for fibronectin transcripts was found. Two papers report endothelial cells to contain collagen a1(III) mRNA. Clement et al reported immunoreactivity for collagen type III in these cells.

SECRETION OF PEPTIDE MEDIATORS

Interleukin 1α

Interleukin 1α is one of the major cytokines participating in the regulation of many immunological and inflammatory responses. In the liver interleukin 1α is produced by...
both endothelial and Kupffer cells. Recent data show that interleukin 1α 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. In addition, culture supernatants of stimulated endothelial cells evoke an acute phase reaction by cultured hepatocytes.

**Kupffer cells**

The ultrastructural characteristics of Kupffer cells are well characterised by electron microscopy (Fig 3). 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 elutration of the resulting cell suspension. Bouwens et al. 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. After partial hepatectomy, the Kupffer cell population is regenerated by local proliferation. 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.

**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.

**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. When stimulated in vitro with lipopolysaccharide, Kupffer cells synthesise tumour necrosis factor α mRNA and secrete the protein in the culture medium. 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.

**Interleukin 1α** - Interleukin 1α is one of the major cytokines participating in the regulation of many immunological and inflammatory responses. In the liver interleukin 1α is produced by both endothelial cells and Kupffer cells. Recent data show that interleukin 1α 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. Kupffer cells in primary culture synthesise interleukin 6. Synthesis can be stimulated by tumour necrosis factor α and interleukin 1.

**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 β1 is currently the best characterised cytokine of this family. This multipotent protein is ubiquitously present in both normal tissues and transformed cells, and almost all cells have receptors for it. It is secreted in inactive form and local factors - that is, lowered pH and proteases may activate the molecule. Besides transforming growth factor β1, four other isoforms are known, transforming growth factor β2-5. Transforming growth factor β1-3 are found in mammals and chickens. Transforming growth factor β4,5 are present only in chickens and amphibians (for a recent review see 114).

Transforming growth factor β is present in conditioned Kupffer cells medium and stimulates collagen synthesis by cultured fat storing cells, while inhibiting proliferation of these cells. It has also an inhibitory effect on the collagen synthesis of parenchymal cells. In freshly isolated cells from normal rat liver, northern hybridisation analysis showed the presence of transforming growth factor β1 mRNA in Kupffer cells and to a lesser extent in fat storing cells. No message is found in hepatocytes or endothelial cells.

**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 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). A number of particulate substances.
and soluble agents, including phosphor ester, zymosan, arachidonic acid, and the calcium ionophore A23187 are known to stimulate in Kupffer cells the formation of prostanooids, mainly prostaglandins prostaglandin D2, prostaglandin E2, and thromboxane. The release of prostaglandins is mainly controlled by a phospholipase A2, which liberates arachidonic acid from membrane lipids. By two different pathways, one involving cyclooxygenase and the other lipo-oxygenase, both prostaglandins and leukotrienes are synthesised starting from arachidonate.

Zymosan and phosphor-12-myristate-13-acetate (PMA) excite superoxide $O_2^-$. Superoxide formation is catalysed by NADPH oxidase residing in the plasma membrane. The superoxide is 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 cocturated hepatocytes. The mediator of this inhibition is probably nitric oxide. It has been shown that the stimulus induced release of prostanooids but not of superoxide depends on the extracellular concentrations of Na+. Na+/H+ exchange and concomitant changes in the cellular pH participated in the stimulus response coupling. Earlier findings showed that calcium ions may also participate in the stimulus induced production and release of prostanooids by Kupffer cells. It has been shown in cell free extracts of rat Kupffer cells that phospholipase A2 was activated at low 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 limited stimulatory effect. 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 acids from phospholipids and suggests hormone induced formation of a protein that modifies the activity of phospholipase A2. Lipocortin is a likely candidate.

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. It has recently been shown that human Kupffer cells also have a tumoricidal action against colon adenocarcinoma in vitro. Generally, Kupffer cells require activation by immunomodulators such as muramyl peptides, lipopolysaccharides, lymphotoxines, and interferon γ. The in vivo role of Kupffer cells was further supported by animal studies where activation and suppression of Kupffer cells affect the number of size of liver metastases after intraportal challenge with tumour cells. Treatment with inhibitors of Kupffer cell function such as silica, gelatinous 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 vivo cytotoxicity of Kupffer cells against colon carcinoma cells was gradually built up during the development of liver metastases. 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.
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