REVIEW

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Recognizing death: liver phagocytosis of apoptotic cells

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SUMMARY

Apoptosis impacts on nearly all areas of cell biology and continues to draw increasing numbers of investigators to join in the multifaceted race to understand it. Within the study of cell death the area that has less benefited from the fast advances has been the study of the phagocytic process of apoptotic cells. But finally this field is now converging the attention and the studies of an increasing number of researchers that are highlighting its importance.

This review deals with removal of apototic cells; in particular, the liver cell mediated removal of apoptotic blood cells will be considered. The involvement of carbohydrate-specific receptors of liver cells in the recognition and engulfment of apoptotic cells has been tested using three different experimental approaches:

i- *in vivo* induction of apoptosis;
ii- *in vitro* phagocytosis;
iii- *in situ* adhesion experiments.

All three main cell liver types are able to recognize and internalize apoptotic cells mainly by means of carbohydrate-specific receptors (galactose and mannose). By up-regulating the cell surface expression of mannose receptors of the endothelial cells, the recognition and the internalization of apoptotic lymphocytes can be increased. Of note is the discrimination in the recognition of apoptotic lymphocytes by the sinusoidal cells: only homologous cells are rapidly and efficiently deleted from the circulation.

I. Liver apoptosis

Apoptosis results in the orderly removal of cells that are defective, no longer needed or otherwise destined to die. However, apoptosis is also the complement of mitosis, and in concert with it determines maintenance, growth or involution of tissue (Gerschenson and Rotello 1991). Although apoptosis occurs at a negligible rate in the normal liver, a variety of physiological conditions, diseases, and xenobiotic treatments can cause this form of cell death. Regression of the liver during starvation is accompained by an enhanced rate of apoptosis (Bursch *et al.* 1992). Cell loss through apoptosis has also been detected in liver during physiological cellular renewal, in cellular depletion after the "overshoot" of cell regeneration of animals subjected to partial hepatectomy (Tessitore *et al.* 1989) and after stimulation with mitogens or hyperplasia-inducing treatments (Columbano *et al.* 1985; Bursch *et al.* 1986). Moreover, apoptosis is also induced by stressful stimuli and by unfavorable enviromental conditions (Columbano *et al.* 1985; Bursch *et al.* 1992; Grasl Kraupp *et al.* 1994; Ledda-Columbano *et al.* 1996) (Fig. 1, Fig. 2).

Apoptosis *in vivo* is followed almost inevitably by rapid uptake into adjacent phagocytic cells. The rapid ingestion of the apoptotic cells before necrosis prevents the leakage of potentially harmful materials and, therefore, limits the potential for inflammatory reactions and auto-immune responses. In fact, the phagocytosis of apoptotic cells,

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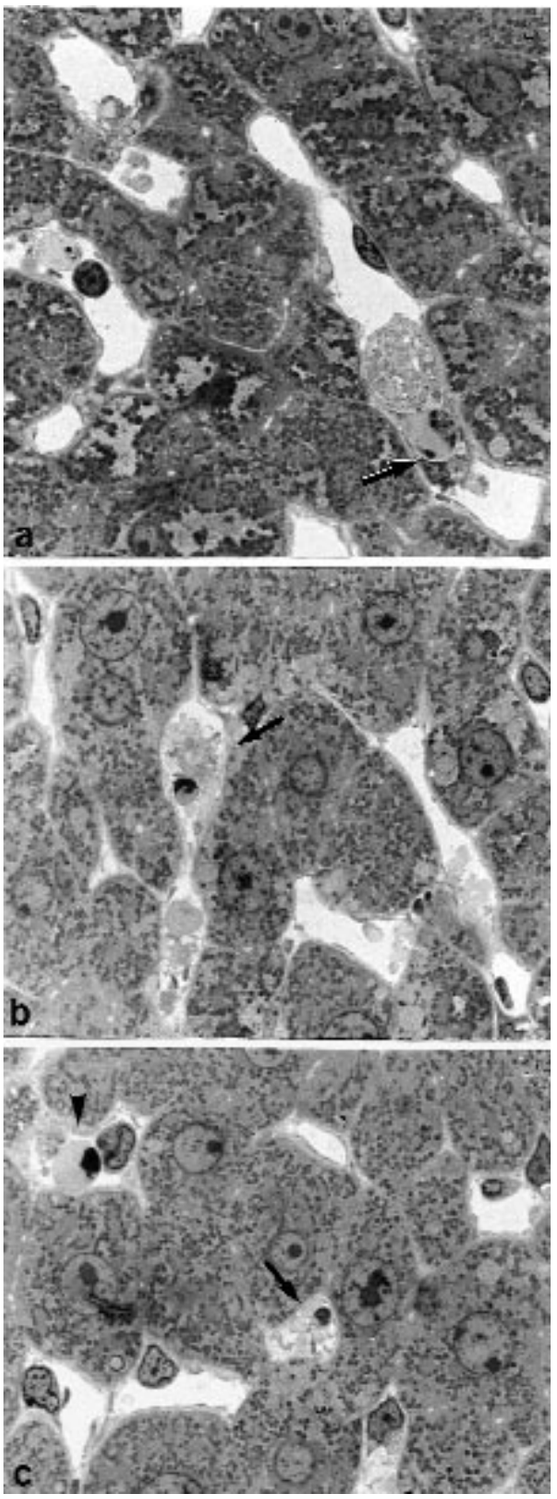

unlike other receptor-mediated phagocytic responses of macrophages, does not lead to the release of pro-inflammatory mediators (Meagher *et al.* 1992; Fadok *et al.* 1998b). In the liver, this function is beautifully performed by liver sinusoidal cells (Dini *et al.* 1996). Due to their location in the sinusoids, and combined with the fact that Kupffer cells represent the majority of the body's fixed macrophages, they are predominant participants in this process, being the first cells of the mononuclear phagocyte system to come into contact with particulate and immunoreactive materials coming from the blood, potentially noxious ones like the apoptotic cells. Very long is the list of the specific receptors that mediate the phagocytic activities of the sinusoidal cells: for example, carbohydrates and glycoproteins (Steer and Clarenburg 1979; Kolb-Bachofen *et al.* 1982; Dini and Kolb-Bachofen 1989), fibronectin, surface-bound fragments of C3 (Wardle 1987; Kempka *et al.* 1990), denaturated proteins and lipids (Nenseter *et al.* 1992; Van Berkel *et al.* 1992), opsonized foreign particles (Kolb-Bachofen 1992), bacteria, yeasts and viruses (Kirn *et al.* 1982), apoptotic bodies (Dini *et al.* 1993) and immune complexes (Wardle 1987), ceruloplasmin (Dini *et al.* 1990), etc.

But what are the mechanisms underlining the phagocytosis of apoptotic cells? And in the liver? For the recognition of apoptotic cells, are specific receptors devoted to this particular function used or shared with others? Even if the molecular mechanisms regulating this process are far from being entirely clarified, an increasing body of evidence indicates that the molecular mechanism by which apoptotic cells are removed is important in impeding the subsequent pro-inflammatory response (Savill *et al.* 1997). Available data have identified candidate phagocyte molecules for restraining apoptotic cells (i.e. lectins, thrombospondin (TPS); CD14; scavenger receptors), transmembrane signalling for phagocytosis ($\alpha_v\beta_3$, CD36, ABC1, an ATP binding Cassette transporter, Ced-6, Ced-7) and cytoskeletal reorganization (Ced-5) (Savill *et*

Fig. 1 - Light micrographs of rat liver 5 days after a single injection of lead nitrate (10mmoles/100 g b.w.). The intoxication with the heavy metal generates apoptotic cells that are visible in (a) inside the sinusoids. (b) and (c) show apoptotic cells phagocytosed by Kupffer cells (arrow). Magnifications (a,b,c) 1200x.

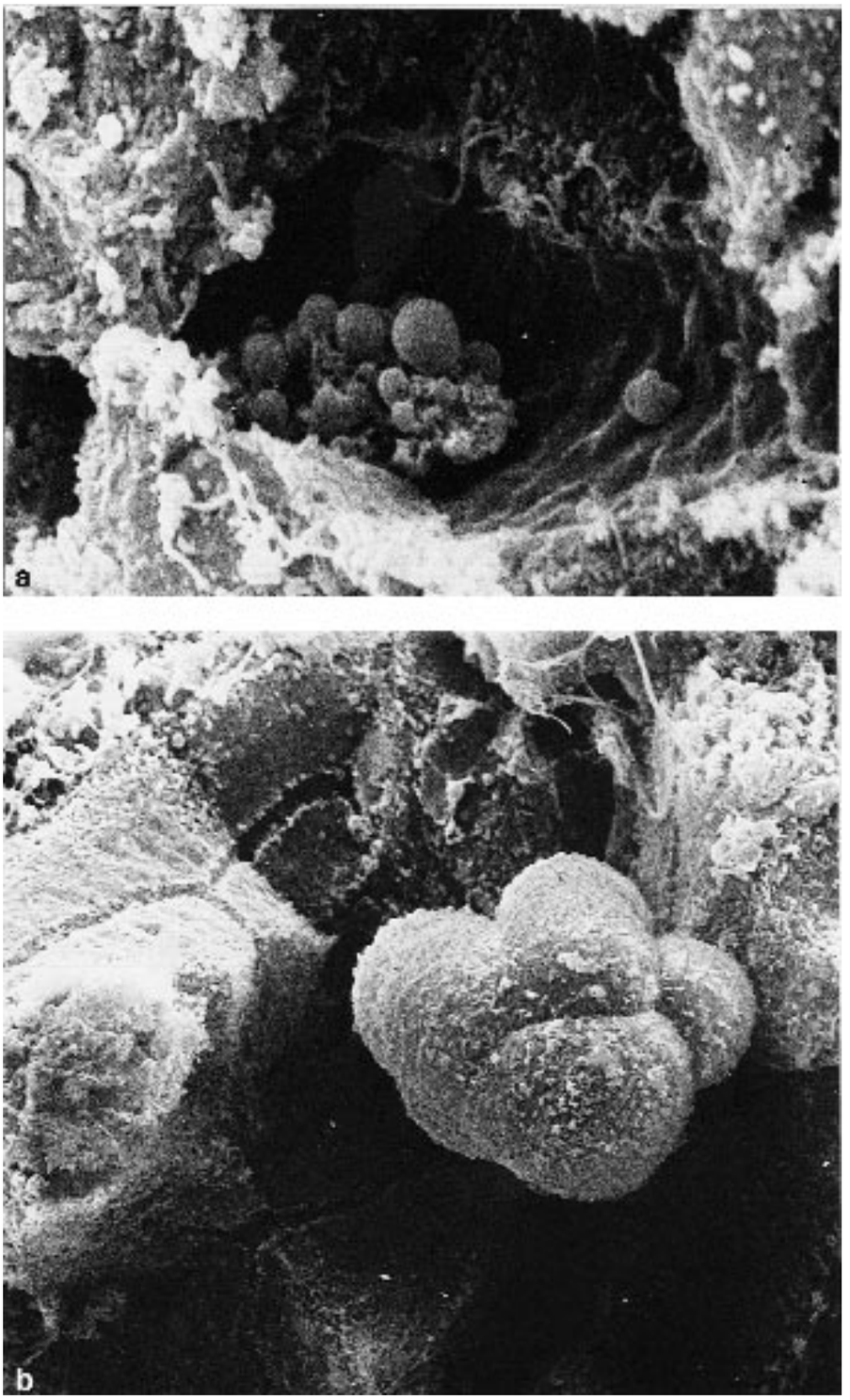

Fig. 2 - Scanning electron micrographs of rat liver 3- (a) and 5 (b) days after lead nitrate administration. (a) Sinusoid with an apoptotic Kupffer cell showing extensive blebbing. (b) An expelled blebbing hepatocyte. Magnifications (a) 3000x; (b) 9000x.

al. 1992; Savill *et al.* 1990; Savill *et al.* 1992; Dini *et al.* 1992; Flora and Gregory 1994; Ren *et al.* 1995; Luciani and Chimini 1996; Devitt *et al.* 1998; Fadok *et al.* 1998a; Liu and Hengartner 1998; Savill 1998; Wu and Horvitz 1998; Schlegel *et al.* 1999).

II. Hepatic lectin-like receptors and liver cell recognition and phagocytosis of apoptotic lymphocytes

Among the several alternative mechanisms reported for removal of apoptotic cells that are mainly related to the cell type and system used, in the liver the recognition and phagocytosis of apoptotic cells are made by means of hepatic lectin-like receptors (Dini *et al.* 1996). The first demonstration that the asialoglycoprotein receptor (ASGP-R) (likely in cooperation with other carbohydrate receptors) is involved in the phagocytosis of apoptotic hepatocytes by healthy ones, was performed on newborn hepatocyte cultures induced to undergo apoptosis by hormonal treatments (Dini et al 1992). The apoptotic bodies, floating in the culture supernatants, were removed by the hepatocytes. The idea that the apoptotic cell surface might expose normally masked sugar residues, rendering them available for interaction with lectin-like receptors on hepatocytes, was supported by the ability of the specific receptor antibodies and sugar moieties to block their binding and uptake by the living liver cells. Therefore, hepatocyte recognition and internalization of apoptotic cells is due to the exposure of several glycans, in particular galactose/N-acetyl-galactosamine, on the surface of apoptotic cells (Dini *et al.* 1992).

Galactose and mannose-terminated particles are cleared from liver circulation by galactose and mannose-specific uptake mechanisms on sinusoidal cells. The galactose-specific receptor shows a high affinity for particulate ligands that expose the galactose group, like desialylated erythrocytes (Kolb-Bachofen *et al.* 1982). Liver endothelial cells utilize galactose-specific receptors for receptor-mediated endocytosis of circulating modified glycoproteins as well as for engulfment of large-sized materials (Steffan *et al.* 1986). Moreover, liver endothelial and Kupffer cells take up a wide range of molecules with a net negative charge by the so-called scavenger receptor (Van Berkel *et al.* 1992) and molecules with mannose- and N-acetyl-glucosamine residues by lectin-like receptors (Steer and Clarenburg 1979). Liver endothelial cells are the primary site for uptake of these glycoproteins when compared with Kupffer cells, which have sixfold lower activity in the uptake of various mannose-exposing ligands from the circulation (Praaning-van Dalen *et al.* 1987). Therefore, since all the three main liver cell types possess receptors that can potentially recognize apoptotic cells, they are predictable actors in the recognition and subsequent engulfment of apoptosing cells.

Three different experimental approaches (i- *in vivo* induction of apoptosis; ii- *in situ* adhesion experiments; iii- *in vitro* phagocytosis) were established to verify the involvement of carbohydrate-specific receptors of liver cells in the recognition and engulfment of apoptotic cells.

i) *in vivo* induction of apoptosis

In spite of the complexity of the phenomena, the investigation of the apoptotic process in the liver *in vivo*, or as a whole *in situ*, enables us to study at the same time different aspects of the process. In particular, the contemporary presence of dying and healthy cells allowed us to verify in the same sample the distribution, the morphology and the modifications of cell surfaces of dying liver cells and the expression of the recognition receptors on the healthy ones. This latter aspect is one of the lesser studied ones at the moment.

Modulation of cell surface molecules has been reported for cells undergoing the process of apoptosis in different experimental conditions (Morris *et al.* 1984; Schlegel *et al.* 1993; Emoto *et al.* 1997; Savill 1998; Fadok *et al.* 1998b) but very little is known about receptor molecules on dying cells or on the neighboring healty ones. Interestingly, on the cell surface of non-apoptotic liver cells (i.e.hepatocytes, Kupffer cells, endothelial cells), the expression of lectin-like receptors (ASGP-R, galactose and mannose-specific receptor) is modulated (enhanced or decreased) during the entire process of apoptosis, induced *in vivo* by administration of a potent liver mitogen, lead nitrate (Dini *et al.* 1993; Dini *et al.* 1995; Ruzittu *et al.* 1999). The number and distribution of binding sites is receptor and cell-type dependent during the days following the metal injection. However, the intensity and the persistence of the modulation are specific for the different liver cell types, thus indicating different (time and modal-

ity) involvement for hepatocytes, Kupffer cells and endothelial cells during the process of apoptosis. It is worth mentioning that a relationship of carbohydrate receptor expression to the differentiated and/or metabolic state of liver cells has been well documented (Massimi *et al.* 1995). Irrespective of the liver cell type during the *in vivo* induction of apoptosis, it was observed that a decrement of galactose binding sites is paralleled by mannose binding site overexpression and *vice versa*. In this way, carbohydrate specific receptors are always expressed in a great amount on the cell surface. The meaning of all the above described changes has to be better understood.

Modification of hepatic membrane composition during apoptosis may be under the control of mitochondria, whose central role in apoptosis has been established (Kroemer 1997). In fact, a single intravenous injection of lead nitrate was able to lower the activity of the mitochondrial tricarboxylate carrier and the lipogenic enzymes as well as to modify the lipid mitochondrial composition, but leaving unaltered the ultrastructure of the mitochondria (Dini *et al.* 1999a). In particular, the reduced activities of cytosolic lipogenic enzymes could suggest a putative mitochondrial control of apoptotic membrane alterations through the tricarboxylate carrier (Dini *et al.* 1999a). In fact, besides other functions, the tricarboxylate carrier plays an important role in fatty acid biosynthesis since it catalyzes the transport of acetyl-CoA, condensed with oxaloacetate in the form of citrate, from mitochondria to the cytosol of the cell, where lipogenesis occurs. Interestingly, Castedo *et al.* (1995) have shown that mitochondrial transmembrane potential disruption leads to phosphatidylserine exposure on the plasma membrane, thus causing alterations of the surface that will facilitate the phagocytic recognition and removal of cells *en route* to apoptosis.

During the dismetabolic state of the liver generated by lead nitrate treatment, sinusoidal liver cells (i.e. Kupffer and endothelial cells) actively phagocyte apoptotic hepatocytes and circulating apoptotic cells, as suggested by ultrastructural analysis. In particular, Kupffer cells at five and fifteen days from the lead nitrate injection internalize apoptotic cells two-three fold over the control (Ruzittu *et al.* 1999). However, phagosomes containing apoptotic hepatocytes are often seen inside the cytoplasm of parenchymal cells and endothelial cells. The ability of endothelial liver cells to recognize and internalize apoptotic cells and/or bodies (Dini *et al.* 1995; Dini and Carlà 1998) is in line with the capacity of the hepatic sinusoidal wall to interact with particulate materials (Steffan *et al.* 1986; Wardle 1987; Dini and Kolb-Bachofen 1989; Kolb-Bachofen 1992) and to operate as a protective barrier for the systemic circulation (Toth and Thomas 1992).

ii) *in vitro* phagocytosis experiments

Liver cells (i.e. hepatocytes, Kupffer cells, endothelial cells as well as pit and fat storing cells) can be dissociated, purified and maintained in suspension or in adhesion cultures for some time (depending on the cell type). Therefore, sinusoidal liver cells are a useful tool for the study of phagocytosis (Fig. 3). *In vitro* adhesion and uptake experiments where performed by using isolated, purified and maintained in adhesion cultures, endothelial and Kupffer cells incubated with apoptotic lymphocytes. The choice of lymphocytes derived from the fact that *in vivo*, apoptotic blood cells are a physiological source of apoptotic cells/bodies recognized and phagocytosed by liver cells. In fact, *in vivo*, apoptotic lymphocytes are recognized and phagocytosed well before the final stages of DNA degradation and cell lysis (Pradham *et al.* 1994; Huang *et al.* 1994).

The recognition process is apparently triggered by modifications of the cell surface (Platt *et al.* 1998; Ren and Savill 1998). On the surface of the apoptotic lymphocytes, fewer varieties of potential ligands have so far emerged, the leading contender being phosphatidylserine (PS), closely followed by carbohydrate changes; other possibilities remain, for the present, on the sidelines. Asymmetric distribution of phospholipids across the bilayer of lymphocyte plasma membrane (maintained by an ATP-dependent aminophospholipid translocase and dissipated by activation of a non-specific lipid flippsite) is lost as part of the program of cell death, by down regulation of the translocase and activation of the non-specific lipid flipsite. As consequence, PS is exposed on the cell surface. In cells in which apoptosis is induced through the Fas system, such as HeLa cells (Shiratsuchi *et al.* 1998), T lymphocytes under activation-induced death (Dhein *et al.* 1995; Brunner *et al.* 1995), acute lymphocytic leukemia cell lines treated with an anti-cancer drug,

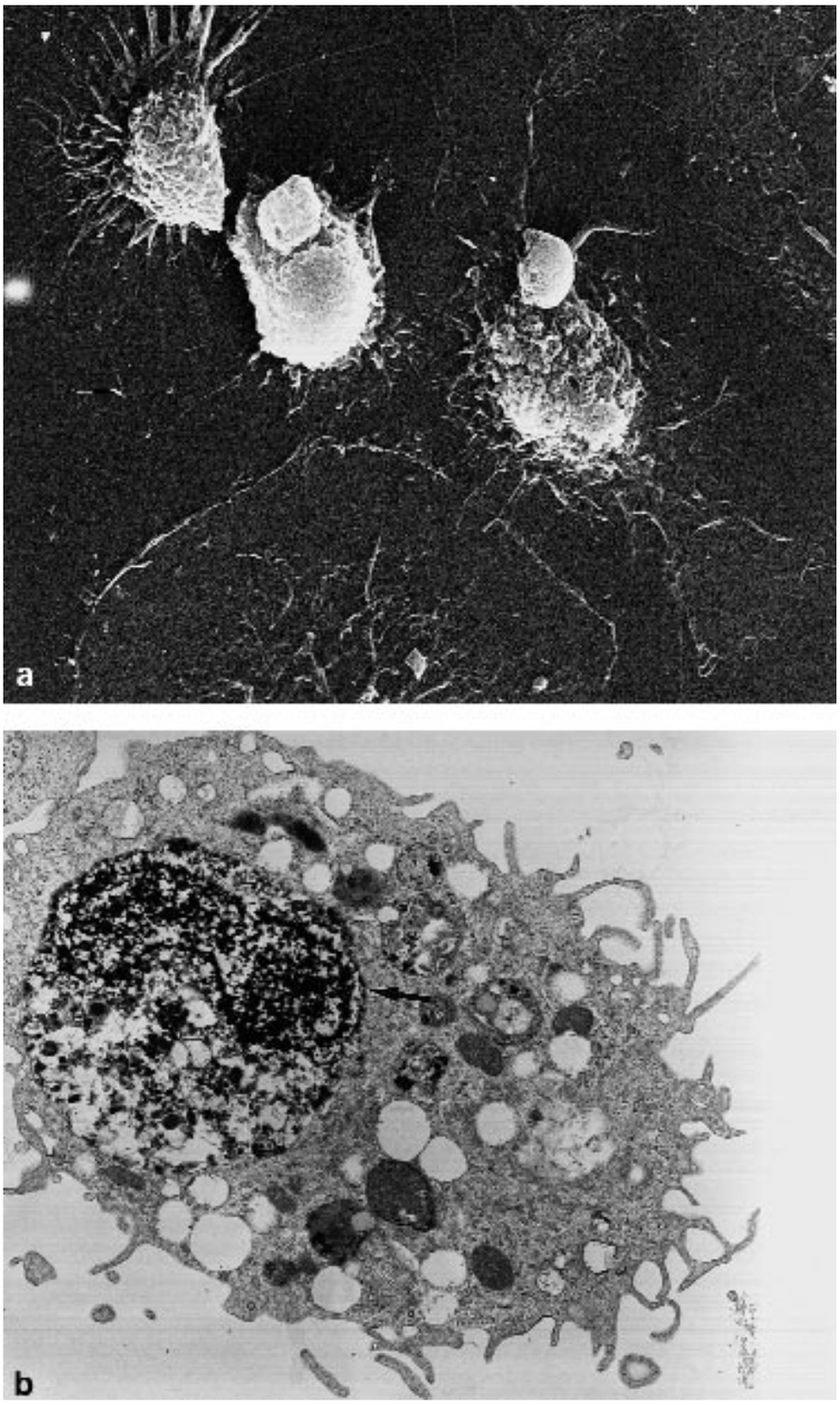

Fig. 3 - Scanning (a) and transmission (b) electron micrographs of the interaction between apoptotic lymphocytes and cultured and isolated Kupffer cells. a) Apoptotic lymphocytes adhering closely to the plasma membrane of Kupffer cells after 5 min of incubation. b) A Kupffer cell with a large phagosome containing still recognizable nuclear dense masses (arrow). Magnifications (a) 2000x; (b) 5000x

doxorubicin (Friesen *et al.* 1996), and influenza virus-infected cultured cells (Fujimoto *et al.* 1998), PS externalization preceded other apoptotic events (Stuart *et al.* 1998). Cells which have lost membrane asymmetry are recognized by macrophages (Mc Evoy *et al.* 1986; Schleger and Williamson 1987; Pradhan *et al.* 1994), but it is still debated whether PS externalization is sufficient for phagocytosis induction. PS externalization independent of apoptosis caused by N-ethylmaleimide treatment leads to PS-mediated phagocytosis, and externalized PS by itself induces apoptosing cell phagocytosis before plasma membrane permeability increases (Shiratsuchi *et al.* 1998). Moreover, that PS exposure has functional consequences demonstrated by the ability of artificial lipid vesicles containing PS to inhibit phagocytosis of apoptotic lymphocytes by macrophages. Understanding the mechanisms that govern membrane lipid sidedness, including those that promote a collapse of phospholipid asymmetry, seems essential to the comprehention of the disease states in which this unwanted PS exposure, or lack of PS exposure, is observed (Kuypers 1998; Verhoven *et al.* 1999).

However, other signals besides PS are also involved in recognition of apoptotic lymphocytes. During our studies aimed to characterize which components of the lymphocyte cell surface underwent modifications during the apoptotic process, we found that the glycidic residues of glycoproteins of plasma membranes were substantially changed in the apoptotic lymphocytes compared to normal cells (Falasca *et al.* 1996). In particular, our binding experiments, using four different fluorescent conjugate-lectins (concanavalin-A, *Phaseolus limensis*, *Ricinus communis* and *Ulex europaeus*) with different hapten sugar specificity, indicate that a relevant amounts of desialylated glycans are exposed on the surface of apoptotic cells. The membranes of apoptotic lymphocytes express increased amount of N-acetyl-galactosamine, D-galactose and mannose residues when compared with normal ones. The same findings were confirmed at the ultrastructural level by labelling apoptotic lymphocytes with gold particle-conjugated lectins (ConA-Au17 and PHA-Au17) that resulted in labelling as small aggregates distributed all over the cell surface of apoptotic cells (Falasca *et al.* 1996). We do not know how these modifications of the cell surface carbohydrates can occur. Probably they are due to the exposure of new membrane derived from the fusion of endoplasmic reticulum or Golgi vesicles during the onset of apoptosis, or they may be due to a possible desialylation process which causes the exposure of normally masked residues (Morris *et al.* 1984). This latter mechanism is responsible for the removal of aged erythrocytes by the liver (Kolb *et al.* 1981).

Kupffer and endothelial cells in culture phagocyte in a very efficient manner lymphocytes undergoing apoptosis, induced by different stimuli (heat-shock 43°C; cycloheximide), but not normal living ones (Falasca *et al.* 1996; Dini and Carlà 1999) (Fig. 3). Phagocytosis is inhibited by the presence in the culture medium of sugars (as single moieties or as a cocktail) and to a lower extent by desialylated glycoproteins, but not by unmodified glycoproteins. The use of single compounds or modified glycoproteins never reaches the level of inhibition achieved by the sugar cocktail, thus suggesting cooperation between galactose and mannose-specific receptors. A difference in the phagocytic activity is easily observed between isolated endothelial and Kupffer cells, the latter being much more active than endothelial cells. The recognition of the apoptotic lymphocytes once added to human Kupffer cell cultures is almost entirely completed within a few minutes of incubation, and the apoptotic cells are detected as dark material inside large phagosomes (Fig. 3b). On the contrary, endothelial cells need more time to complete engulfment of apoptotic lymphocytes. One explanation is of course related to the different functions in the liver of endothelial and Kupffer cells, which being macrophages are characterized by the phagocytic activity. However, it is worth noting that also the state of the phagocyte is particularly important in apoptotic recognition (Savill *et al.* 1993). The particular mechanism employed by macrophages and/or other immature phagocytes may be regulated by external influences. The exposure of human monocyte-derived macrophages to granulocyte-macrophage colony stimulating factor (GM-CSF), a cytokine known to be present at inflammation sites, increased the recognition of apoptotic human neutrophils (Savill *et al.* 1993). Cytokine implicated in repair of injured tissue (i.e. transforming growth factor, TGF-β; platelet-derived growth factor, PDGF)

and those involved in the initiation of inflammation (i.e. interferon gamma, IFN-γ; interleukin-1, IL-1 and tumor necrosis factor-α; TNF-α) also stimulated TPS-dependent recognition of apoptotic neutrophils (Savill *et al.* 1993). IL-5 modulates macrophage phagocytosis of apoptotic eosinophils (Stern *et al.* 1992). LPS and IL1b up regulate the mannose receptor expression of liver cells and consequently the phagocytic activity of sinusoidal cells (Dini *et al.* 1995).

iii) *in situ* adhesion experiments

The use of *in situ* adhesion experiments highlights the role of the cell surface modifications on dying cells and of lectin-like receptors (in particular galactose- and mannose-specific receptors) in the liver recognition of dead cells (Ruzittu *et al.* 1999). Once injected into the mouse hepatic circulation, apoptotic lymphocytes are efficiently removed by the sinusoidal cells. This removal is mediated by the carbohydrate receptors, as confirmed by inhibition studies (Dini *et al.* 1995; Dini 1998; Dini *et al.* 1999). Conversely, non apoptotic cells, are not tethered by sinusoidal liver cells.

Interestingly, apoptotic lymphocytes are retained by the sinuoids in a heterogeneic distribution: apoptotic cells in the periportal tract are double those in the perivenous region (Dini and Carlà 1998). The reason should be found in the differences existing between periportal and centrilobular endothelial cells regarding the fenestration pattern (Morin *et al.* 1984) and to the uneven expression of galactose and mannose-specific receptors (Rocha *et al.* 1993). Mannose receptor expression on the liver endothelium is up-regulated by IL-1 and is associated with increased removal of apoptotic cells and tumor cell adhesion (Vidal-Vanaclocha *et al.* 1994; Dini *et al.* 1995; Asumendi *et al.* 1996). The ability to recognize apoptotic lymphocytes has, therefore, been related to the amount of carbohydrate receptors expressed on the cell surface (Dini and Carlà 1998). Unexpectedly, apoptotic U937 cells (a monocytic cell line) are recognized by Kupffer and endothelial cells only to a very low extent (Dini *et al.* 1999b). One possible explanation could derive from the fact that, as surface lectin and Annexin V binding is concerned, the cell surface of apoptotic U937 cells is not significantly different from the healthy ones (Dini *et al.* 1999b).

Our current studies are demonstrating that on apoptotic cells the glycan modifications are progressively achieved and are paralleled by the morphological modifications. Therefore, as cell surface modifications are concerned, it is possible to divide the execution process of apoptosis in three stages: early, mature and late/necrotic. By using these isolated different stages of apoptotic cells in our adhesion experiments, we observed a higher recognition for the "mature" apoptotic cells by the sinsoidal wall, while the late/necrotic apoptotic cells are recognized to the lower extent (manuscript in preparation).

Analysis of cell surface glycoconjugates between normal and apoptotic lymphocytes isolated from different species (i.e. human, rat), show that cell surface modifications of lymphocytes undergoing apoptosis are species related. In fact, DBA binding is detectable only on rat apoptotic lymphocytes while LPA binds on human apoptotic lymphocytes. Moreover, PS is differently expressed on dying rat and human lymphocytes. Rat apoptotic lymphocytes exhibit a higher intensity of Anne in V-FITC binding than human apoptotic lymphocytes. These differences are responsible for the different rates of removal and internalization by murine sinusoidal liver cells. In fact, human apoptotic lymphocytes are removed very efficiently by the sinusoidal wall cells, while the same cells recognize and internalize to a lesser extent (about 30%) rat apoptotic lymphocytes (Dini 1998).

Concluding remarks

The previous brief discussion of the recognition and ingestion of apoptotic cells by liver cells shows clearly that liver cells are active participants in the removal of apoptotic cells and that this removal is swift and efficient. Phagocytosis of apoptotic cells by liver cells is a complex phenomena as shown by the presence of multiple molecular mechanism(s) of recognition. The recognition of dead cells could be a multi-step process complicated by the existence of regional specialization and by the display on the apoptotic cells of multiple signals to increase the probability of their removal and consequently the safety for the whole organism. In addition, the removal of apoptotic lymphocytes by liver is an example of co-operation among different cellular types sharing the same receptor system. In fact, hepatocytes, Kupffer cells and endothelial cells perform the

plasma clearance of apoptotic cells generated during the involuting phase of liver hyperplasia induced by a single injection of lead nitrate by means of a sugar recognition mechanism (Dini *et al.* 1994, 1995). These data, together with the fact that the phagocytic activity in endothelial cells can be enhanced in macrophage-depleted rats (Bogers *et al.* 1991) and that IL-1 induces *in vitro* overexpression of mannose-specific receptors on endothelial cells, further support the idea of cooperation among liver cells during phagocytosis of apoptotic cells (Dini *et al.* 1996; Dini 1999). However, a novel peculiarity is emerging in the complex field of the process of liver phagocytosis of apoptotic cells. In fact, even if all the cells undergoing apoptosis exhibit cell surface modifications, these are different in the same cell type of different species (i.e. rat and human lymphocytes). Therefore, the process of phagocytosis of apoptotic cells, which is an ancient process present in Invertebratres as well as in Vertebrates, has developed species-specific mechanisms, whose biological significance is still obscure.

Further investigations the elucidation of the molecular basis for recognition of apoptosing cells by phagocytes are necessary for a better understanding of the fate of apoptotic cells, that in turn lead to the development of new therapeutics to overcome diseases for which effective medical treatment is not yet available; for example, whether compromising the capability to ingest apoptosing cells contributes to autoantibody production (Voll *et al.* 1997; Fadok et al 1998b; Botto *et al.* 1998; Herrmann *et al.* 1998).

REFERENCES

Asumendi A., Alvarez A., Martinez I., Smedsrod, B., and Vidal-Vanaclocha, F.: Hepatic sinusoidal endothelium heterogeneity with respect to mannose receptor activity is interleukin 1 dependent. Hepatology *23*, 1521-1529, 1996.

Bogers W.M., Stad R.K., Janssen D.J., Prins F.A., Van Rooijen N., Van Es L.A., and Daha M.R.: Kupffer cell depletion *in vivo* as results in clearance of large-sized IgA aggregates in rats by liver endothelial cells. Clin. Exp. Immunol. *85*, 128-136, 1991.

Botto M., Dell'Agnola C., Bygrave A.E., Thompson E.M., Cook H.T., Petry F., Loos M., Pandolfi P.P., and Walport M.J.: Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. Nat. Genet. *19*, 56-59, 1998.

Brunner T., Mogil R.J., LaFace D., Yoo N.J., Mahboubi A., Echeverri F., Martin S.J., Force W.R., Lynch D.H., Ware C.F., and Green D.R.: Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. Nature *373*, 441-444, 1995.

Bursch W., Dusterberg B., and Schulte-Hermann R.: Growth, regression and cell death in rat liver as related to tissue levels of the hepatomitogen cytoproterone acetate. Arch. Toxicol. *59*, 221-227, 1986.

Bursch W., Oberhammer F., and Schulte-Hermann R.: Cell death by apoptosis and its protective role against disease. Trends Pharmacol. Sci. *13*, 245-251, 1992.

Castedo M., Macho A., Zamzami N., Hirsh T., Marchetti P., Uriel J., and Kroemer G.: Mitochondrial perturbations define lymphocytes undergoing apoptotic depletion *in vivo*. Eur. J. Immunol. *25*, 3277-3284, 1995.

Columbano A., Ledda-Columbano G.M., Coni P., Faa G., Liguori C., Santacruz G., and Pani G.: Occurrence of cell death (apoptosis) during the involution of liver hyperplasia. Lab. Invest. *52*, 670-677, 1985.

Devitt A., Moffatt O.D., Raykundalia C., Capra J.D., Simmons D.L., and Gregory C.D.: Human CD 14 mediates recognition and phagocytosis of apoptotic cells. Nature *392*, 505-508, 1998.

Dhein J., Walczak H., Baumler C., Debatin K.M., and Kramer P.H.: Autocrine T-cell suicide mediated by APO-1/. Nature *373*, 438-441, 1995.

Dini L., and Kolb-Bachofen V.: Preclustered receptor arrangement is a prerequisite for galactose-specific clearance of large particulate ligands in rat liver. Exp Cell Res *184*, 235-240, 1989.

Dini L. Carbonaro M., Musci G., and Calabrese L.: The interaction of ceruloplasmin with Kupffer cells. Europ. J. Cell Biol. *52*, 207-212, 1990.

Dini L., Autuori F., Lentini A., Oliverio S., and Piacentini M.: The clearance of apoptotic cells in the liver is mediated by the asialoglycoprotein receptor. FEBS Lett . *296*, 174-178, 1992.

Dini L., Falasca L., Lentini A., Mattioli P., Piacentini M., Piredda L., and Autuori F.: Galactose-specific receptor modulation related to the onset of apoptosis in rat liver. Europ. J. Cell Biol. *61*, 329-337, 1993.

Dini L., Lentini A., Diez Diez G., Rocha M., Falasca L., Serafino L., and Vidal-Vanaclocha F.: Phagocytosis of apoptotic bodies by liver endothelial cells. J. Cell Sci. *108*, 967-973, 1995.

Dini L., Ruzittu M., and Falasca L.: Recognition and phagocytosis of apoptotic cells. Scanning microscopy *10*, 239-252, 1996.

Dini L., and Carlà E.C.: Hepatic sinusoidal endothelium heterogeneity with respect to the recognition of apoptotic cells. Exp. Cell Res. *240*, 388-393, 1998.

Dini L.: Endothelial liver cell recognition of apoptotic peripheral blood lymphocytes. Biochem. Soc. Transact. *26*, 635-639, 1998.

Dini L., Giudetti A.M., Ruzittu M., Gnoni G.V., and Zara V.: Citrate carrier and lipogenic enzyme activities in lead nitrate-induced proliferative and apoptotic phase in rat liver. Biochim. Mol. Biol. Intern. *47*, 607-614, 1999a.

Dini L., Carlà E.C., De Luca M., Faraldi G., and Tagliafierro G.: Phagocytosis of apoptotic cells: liver recognition and molecular mechanisms. Ital. J. Zoology *66*, 317-322, 1999b.

Emoto K., Toyama-Sorimachi N., Karasuyama H., Inoue K., and Umeda M.: Exposure of phosphatidylethanolamine on the surface of apoptotic cells. Exp. Cell Res. *232*, 430-434, 1997.

Fadok V.A., Bratton D.L., Frasch S.C., Warner M.L., and Henson P.M.: The role of phosphatidylserine in recognition of apoptotic cells by phagocytes. Cell Death Differ. *5*, 551-562, 1998a.

Fadok V.A., Bratton D.L., Konoval A., Freed P.W., Westcott J.Y., and Henson P.M.: Macrophages that have ingested apoptotic cells *in vitro* inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-b, PGE2, and PAF. J. Clin. Invest. *101*, 890-898, 1998b.

Falasca L., Bergamini A., Serafino A., Balabaud C., and Dini L.: Human Kupffer cell recognition and phagocytosis of apoptotic peripheral blood lymphocytes. Exp. Cell Res. *224*, 152-162, 1996.

Flora P.K., and Gregory C.D.: Recognition of apoptotic cells by human macrophages: inhibition by a monocyte/macrophage-specific monoclonal antibody. Eur. J. Immunol. *24*, 2625-2632, 1994.

Friesen C., Herr I., Krammer P.H., and Debatin K.M.: Involvement of the CD95 (APO-1/Fas) receptor/ligand system in drug-induced apoptosis in leukemia cells. Nature Med. *2*, 574-577, 1996.

Fujimoto I., Takizawa T., Ohba Y., and Nakanishi Y.: Co-expression of Fas and Fas ligand on the surface of influenza virus-infected cells . Cell Death Differ. *5*, 426-431, 1998.

Gerschenson L.E., and Rotello R.J.: Apoptosis and cell proliferation are terms of the growth equation. In: Apoptosis. The molecular basis of cell death. Cold Spring Harbor Laboratory Press p. 175, 1991.

Grasl Kraupp B., Bursch W., Ruttkay Nedecky B., Wagner A., Lauer B., and Schulte-Hermann R.: Food restriction eliminates preneoplastic cells through apoptosis and antagonizes carcinigenesis in rat liver. Proc. Natl. Acad. Sci. USA *91*, 9995-9999, 1994.

Herrmann M., Voll R.E., Zoller O.M., Hagenhofer M., Ponner B.B., and Kalden J.R.: Impaired phagocytosis of apoptotic cell material by monocyte-derived macrophages from patients with systemic lupus erythematosus. Arthritis Rheum. *41*, 1241-1250, 1998.

Huang L., Soldevila G., Leeker M., Flavell R., and Crispe N.: The liver eliminates T cells undergoing antigen-triggered apoptosis in vivo. Immunity *1*, 741-749, 1994.

Kempka G., Roos P., and Kolb-Bachofen V.: A membrane-associated form of C-reactive protein is the galactose-specific particle receptor on rat Kupffer cells. J. Immunol. *144*, 1004-1009, 1990.

Kirn A., Gut J.P., and Gendrault J.L.: Interaction of viruses with sinusoidal cells. In: Popper H, Schaffner F (eds) Progress in liver diseases. New York: Grune & Stratton, p. 377, 1982.

Kolb H., Friedrick E., and Suss R.: Lectin mediates homing of neuraminidase-treated erythrocytes to the liver as revealed by scintigraphy. Hoppe-Seyler's Z Physiol. Chem. *362*, 1609-1614, 1981.

Kolb-Bachofen V.: A review on the biological properties of C-reactive protein. Immunobiol. *183*, 133-145, 1992.

Kolb-Bachofen V., Schlepper-Schafer J., and Vogell W.: Electron microscopic observations of the hepatic microscopic evidence for an asailoglycoprotein receptor on Kupffer cells: localization of lectin mediated endocytosis. Cell *29*, 859-866, 1982.

Kroemer G.: Mitochondrial implication in apoptosis. Towards an endosymbiont hypothesis of apoptosis evolution. Cell Death Differ. *4*, 443-456, 1997.

Kuypers F.A.: Phospholipid asymmetry in health and disease. Curr. Opin. Hematol. *5*, 122-131, 1998.

Ledda-Columbano G.M., Shinozuka H., Katyal S.L., and Columbano A.: Cell proliferation, cell death and hepatocarcinogenesis. Cell Death Differ. *3*, 17-22, 1996.

Liu Q.A., and Hengartner M.O.: Candidate adaptor protein CED-6 promotes the engulfment of apoptotic cells in C.elegans. Cell *93*, 961-972, 1998.

Luciani M.F., and Chimini G.: The ATP binding cassette transporter ABCD1, is required for the engulfment of corpses generated by apoptotic cell death. Embo J. *15*, 226-235, 1996.

Massimi M., Conti Devirgiliis L., Kolb-Bachofen V., and Dini L.: Independent modulation of galactose-specific receptor expression in rat liver cells. Hepatology *22*, 1819-1828, 1995.

McEvoy L., Williamson P., and Schlegel R.A.: Membrane phospholipid asymmetry as a determinant of erytrocyte recognition by macrophages. Proc. Natl. Acad. Sci. USA *83*, 3311-3315, 1986.

Meagher L.C., Savill J.S., Baker A., Fuller R., and Haslett C.: Phagocytosis of apoptotic neutrophils does not induce macrophage release of thromboxane B2. J. Leuk. Biol. *52*, 269-273, 1992.

Morin O., Patry P., and Lafleur L.: Heterogeneity of endothelial cells of adult rat liver as resolved by sedimentation velocity and flow cytometry. J. Cell Physiol. *119*, 327-334, 1984.

Morris R.G., Hargreaves A.D., Duvall E., and Wyllie A.H.: Surface changes in thymocytes undergoing apoptosis. Am. J. Path. *115*, 426-436, 1984.

Nenseter M.S., Gudmundsen O., Roos N., Maelandsmo G., Drevon C.A.,and Berg T.: Role of liver endothelial and Kupffer cells in clearing low density lipoprotein from blood in hypercholesterolemic rabbits. J. Lipid. Res. *33*, 867-77,1992.

Platt N., Pedro da Silva R., and Gordon S.: Recognizing death: the phagocytosis of apoptotic cells. Trends in Cell Biology *8*, 365-372, 1998.

Pradhan D., Williamson P., and Schlegel R.A.: Phosphatidylserine vesicles inhibit phagocytosis of erythrocytes with a symmetric transbilayer distribution of phospholipids. Mol. Membr. Biol. *11*, 181-187, 1994.

Praaning-van Dalen D.P., de Leeuw A.M., Brouwer A., and Knook D.L.: Rat liver endothelial cells have a greater capacity than Kupffer cells to endocytose N-acetylglucosamine- and mannose- terminated glycoproteins. Hepatology *7*, 672-679, 1987.

Ren V., Silverstein R.L., Allen J., and Savill J.S.: CD36 gene transfer confers capacity for phagocytosis of cells undergoing apoptosis. J. Exp. Med. *181*, 1857-1862, 1995.

Ren V., and Savill J.: Apoptosis: the importance of being eaten. Cell Death Differ. 5, 563-568, 1998.

Rocha M., Lentini A., Asumendi A., Falasca L., Autuori F., Dini L., and Vidal-Vanaclocha F.: *In situ* and *in vitro* correlation between mannose receptor expression and fenestration pattern in endothelial cells selected from different zones of liver lobule. In Knook D, Wisse E. (ed) Cells of the hepatic sinusoid, vol 5. Kupffer Cell Foundation, Leiden, The Netherlands p. 470, 1993.

Ruzittu M., Carlà E.C., Montinari M.R., Maietta G., and Dini L.: Modulation of cell surface expression of liver carbohydrate receptors during *in vivo* induction of apoptosis with lead nitrate Cell Tissue Res. *298*, 105-112, 1999.

Savill J.S., Henson P.M., and Haslett C.: Phagocytosis of aged human neutrophils by macrophages is mediated by a novel "charge-sensitive" recognition mechanism. J. Clin. Invest. *84*, 1518-1527, 1989.

Savill J.S., Dransfield L., Hogg N., and Haslett C.: Vitronectin receptor-mediated phagocytosis of cells undergoing apoptosis. Nature *343*, 170-173, 1990.

Savill J.S., Hogg N., Ren Y., and Haslett C.: Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophilis undergoing apoptosis. J. Clin. Invest. *90*, 1513-1522, 1992.

Savill J.S., Fadok V., Henson P., and Haslett C.: Phagocyte recognition of cells undergoing apoptosis. Immunol. Today *14*, 131-136, 1993.

Savill J.S.: Recognition and phagocytosis of cells undergoing apoptosis. Br. Med. Bull. *53*, 491-508, 1997.

Savill J.S.: Phagocytic docking without shocking. Nature 392, 442-443, 1998.

Schlegel R.A., and Williamson P.: Membrane phospholipid organization as a determinant of blood cell-reticuloendothelial cell interactions. J. Cell. Physiol. *132*, 381-384, 1987.

Schlegel R.A., Stevens M., Lumley-Sapanski K., and Williamson P.: Altered lipid packing identifies apoptotic thymocytes. Immunol. Lett. *36*, 283-288, 1993.

Schlegel R.A., Krahling S., Callahan M.K., and Williamson P.: CD14 is a component of multiple recognition systems used by macrophages to phagocytose apoptotic lymphocytes. Cell Death Differ. *6*, 583-592, 1999.

Shiratsuchi A., Osada S., Kanazawa S., and Nakanishi Y.: Essential role of phosphatidylserine externalization in apoptosing cell phagocytosis by macrophages. Biochem. Biophys. Res. Commun. *246*, 549-555, 1998.

Steer C.J., and Clarenburg R.: Unique distribution of glycoprotein receptors on parenchymal and sinusoidal cells of rat liver. J. Biol. Chem. *254*, 4457-4461, 1979.

Steffan A.M., Gendrault J.L., McCuskey R.S., McCuskey P.A., and Kirn A.: Phagocytosis, an unrecognized property of murine endothelial liver cells. Hepatology *6*, 830-836, 1986.

Stern M., Meagher L., Savill J.S., and Haslett C.: Apoptosis in human eosinophils. Programmed cell death in the eosinophil leads to phagocytosis by macrophages and is modulated by IL-5. J. Immunol. *148*, 3543-3549, 1992.

Stuart M.C., Damoiseaux J.G., Frederik P.M., Arends J.W., and Reutelingsperger C.P.: Surface exposure of phosphatidylserine during apoptosis of rat thymocytes precedes nuclear changes. Europ. J. Cell Biol. *76*, 77-83, 1998.

Tessitore L., Valente G., Bonelli G., Costelli P., and Baccino F.M.: Regulation of cell turnover in the livers of tumor bearing rats: occurrence of apoptosis. Int. J. Cancer *44*, 697-700, 1989.

Toth C.A., and Thomas P.: Liver endocytosis and Kupffer cells. Hepatology *16*, 255-266, 1992.

Van Berkel T.J., De Rijke J.B., and Kruijt J.K.: Recognition of modified lipoprotein by various scavenger receptors on Kupffer and endothelial liver cells. In: Windler E, Greten H, eds. Hepatic endocytosis of lipids and proteins. Munchen, FRG: Zuckschwerdt Verlag p. 443, 1992.

Verhoven B., Krahling S., Schlegel R.A., and Williamson P.: Regulation of phosphatidylserine exposure and phagocytosis of apoptotic T lymphocytes. Cell Death Differ. *6*, 262-270, 1999.

Vidal-Vanaclocha F., Amezaga C., Asumendi A., Kaplanski G., and Dinarello C.A.: Interleukin-1 receptor blockade reduces the number and size of murine B16 melanoma hepatic metastases. Cancer Res. *54*, 2667-2672, 1994.

Voll R.E., Hermann M., Roth E.A., Stach C., Kalden J.R., and Girkontaite I.: Immunosuppressive effects of apoptotic cells. Nature *390*, 350-351, 1997.

Wardle E.M.: Kupffer cells and their function. Liver *7*, 63-70, 1987.

Wu Y.C., and Horvitz H.R.: C.elegans phagocytosis and cell-migration protein CED-5 is similar to human DOCK 180. Nature *392*, 501-504, 1998.