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Review Article

Recognition by Macrophages and Liver Cells of Opsonized Phospholipid Vesicles and Phospholipid Headgroups

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The interaction of liposomes with blood proteins is believed to play a critical role in the clearance pharmacokinetics and tissue distribution of intravenously injected liposomes. In this article we have focused our discussion on the interaction of liposomes with key blood proteins, which include immunoglobulins, complement proteins, apolipoproteins, fetuin, von Willebrand factor, and thrombospondin, and their role in liposome recognition by professional phagocytes and nonmacrophage hepatic cells. Alternatively, macrophages as well as hepatocytes and liver endothelial cells may phagocytose/endocytose liposomes via direct recognition of phospholipid headgroups. A number of plasma membrane receptors such as lectin receptors, CD14, various classes of scavenger receptors (e.g., classes A, B, and D), FcγRI and FcγRII-B2 may participate in phospholipid recognition. These concepts are also discussed.

KEY WORDS: liposomes; Kupffer cells; macrophage; opsonins; phosphatidylserine; complement activation; complement receptors; scavenger receptors.

INTRODUCTION

Recovery of liposomes from the blood or separation of liposomes from *in vitro* serum or plasma incubations has demonstrated that liposomes acquire a coating of proteinaceous molecules (1). However, the binding of blood proteins to liposomes differs considerably in amount and in pattern depending on the biophysical properties of the vesicles and other factors. These include vesicle morphology, surface curvature and charge, lipid composition, bilayer packing, temperature-dependent packing defects, and vesicle dose, as well as the methods used for isolating liposomes from the blood or serum (2–5). Studies of blood protein-liposome interactions have begun to rationalize the clearance pharmacokinetics and tissue distribution of intravenously injected vesicles in different animal models. If such protein-lipid interaction studies are indicative of liposome clearance profiles and their final destination, then it is necessary to identify the key blood proteins and determine their mode of action. In addition, it is also necessary to evaluate how the protein binding profile changes over time for liposome compositions having relatively long circulation times or following repeated vesicle administration. For example, a recent study (6) demonstrated that intravenous administration of poly(ethylene glycol)-grafted liposomes (an example of long circulating vesicles) into rats could elicit the production of a serum factor that influences pharmacokinetics and Kupffer cell recognition of subsequent liposome injections. Interpretation of the *in vitro* observations should also be done cautiously. For example, differences have been reported in the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profile of the proteins associated with various anionic large unilamellar vesicles (LUVs) isolated *in vivo* compared with vesicles isolated from *in vitro* incubations, thus reflecting the complexity associated with the *in vivo* system. For instance, two proteins with apparent molecular weights of 22 and 14 kDa were found to be associated with LUVs *in vivo,* but were absent from recovered LUVs *in vitro* (4). Some of these proteins may represent cell-derived proteins and/or proteolytic fragments generated from physiologically vital protein-protease systems (e.g., blood coagulation systems) and could play a vital role in vesicle recognition by phagocytic cells.

In this minireview, we discuss recent breakthroughs in liposome blood protein interaction and recognition of such complexes by macrophages and other relevant cells. We also speculate on the role of some ignored blood proteins on vesicle opsonization. Nonopsonic blood proteins could also play an important role in particle clearance. Following adsorption onto particle surfaces, nonopsonic proteins could experience conformational changes. Such changes probably expose chemical groups that could either be recognized directly by certain phagocyte cell surface receptors or could act as ligands for subsequent recognition by blood opsonins. On the other hand, macrophages and endothelial cells may directly recognize phospholipid headgroups leading to vesicle endocytosis/phagocytosis. We also discuss the involvement of possible receptors in this process.

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OPSONIZATION AND OPSONORECOGNITION OF LIPOSOMES

Immunoglobulins

Liposomes can be intentionally opsonized with nonspecific and monoclonal antibodies in order to enhance their recognition and clearance by macrophages via their Fc receptors both *in vitro* and *in vivo*. This subject has been reviewed repeatedly in the past (7). *In vivo* opsonization of liposomes by IgM and different subclasses of IgG can also occur. For example, interaction of natural antibodies against liposomal cholesterol and phospholipids may play a critical role in vesicle clearance from the blood, not only via macrophage Fc receptors but also by incorporation with complement and apolipoprotein receptors (see also complement and apolipoproteins) (8,9). In the absence of complement, the clearance of IgG-opsonized particles by macrophages involves binding to Fc receptors, engulfment by lamellipodia that project from the cell surface, internalization by a zipper process, and subsequent delivery of the particles to acidic endosomes and finally to lysosomes for degradation (10). Furthermore, the size of immunoliposomes plays a critical factor in determining the extent of vesicle entry into the cell; the smaller the size of the immunoliposomes, the greater their uptake (reviewed in reference 7). IgG-mediated phagocytosis of drug carriers is associated with the loss of the cell surface Fc receptor. This down-regulation of Fc receptors arises when the receptor is not recycled to the plasma membrane, but instead is degraded in lysosomes. Recycling of Fc receptors takes place only when a monovalent ligand is bound to the receptor (10).

A number of Fc receptors could participate in recognition of antibody-bearing vesicles. The human FcγRI (homologous to murine FcRI or the monomeric IgG2a receptor) is not only the high-affinity receptor for monomeric IgG, but also recognizes IgG-coated particles (11) (see also macrophage FcγRII-B2 in this review). This receptor is unique among the Fcγ receptors in being confined to resting cells of a single lineage, the phagocytic monocyte/macrophage (12). However, resting human Kupffer cells do not express FcγRI (12). In the liver, expression of FcγRI, is restricted to Kupffer cells at the sites of inflammation and is apparently unrelated to the type of liver disease (12). This receptor may play some role in liposome clearance in individuals suffering from liver diseases (see also Macrophage FcγRII-B2 section, this review). The recognition of IgG-coated particles by resident liver and spleen macrophages is usually confined to FcγRIIIA (11,12). In human liver, the expression of the FcγRIIIA is restricted only to Kupffer cells located in the central area of the liver lobule, and its expression is enhanced in patients with acute and chronic active hepatitis (types B and C) (12). The Fc receptors found on monocytes and lymphocytes also seem to participate in the recognition of C-reactive protein-coated vesicles (7,10,11).

Complement Proteins

Liposomes have been used extensively as a model membrane to study the mechanism of complement activation and complement-mediated membrane damage. However, conflicting reports have emerged concerning the important physicochemical properties of liposomes for complement activation and the relevance of such information with regard to *in vivo* liposome pharmacokinetics and clearance in different mammals. The basis of these inconsistencies is due to either species/intraspecies differences in complement proteins and complement activation and/or to the artificial nature of the *in vitro* studies (e.g., the absence of vascular shear forces, exposure to anticoagulated plasma or serum, type of the anticoagulant, or premature degradation of some complement proteins).

Nevertheless, liposomal activation of the classical pathway occurs when natural antibodies to phospholipids and cholesterol bind to the vesicles (5,8,9,13,14). Such antibodies are widespread in all animal species, although specificities and titers show substantial inter- and intraspecies variation (8,14). Liposomes can also activate complement through nonantibody-mediated mechanisms via the classical and alternative pathways (5,13,15,16). For example, in the absence of specific antibodies, anionic phospholipids such as cardiolipin, phosphatidylserine (PS), phosphatidic acid (PA), dicetyl phosphate (DCP), and phosphatidylglycerol (PG) when incorporated into the liposome bilayer interact with the complement protein C1q, a process which leads to activation of the classical pathway in rat, guinea pig, and human (13,17,18). In human serum, PS also activates complement via the alternative pathway; this activation is further enhanced by incorporation of phosphatidylethanolamine (PE) into the vesicular bilayer (7). Similarly, liposomes prepared from lipid extracts of the inner membrane leaflet of erythrocytes (enriched with PS and PE) or having a composition similar to that of sickle red blood cell outer leaflet (enriched with PE) all activate the human complement system via the alternative pathway. The presence of positively charged lipids, particularly those that have been used in gene-transfer protocols, promotes complement activation by the alternative pathway in human serum. However, some positively charged vesicles activate the classical pathway following binding to C-reactive protein (CRP) (19). Note that in rat serum, alternative pathway activation by cationic lipids is minimal (20). Complement activation may also occur following association of serum mannose-binding protein (MBP), a C-type lectin with specificity for mannose and *N*-acetylglucosamine sugars, with the liposome surface. For example, MBP is known to bind to certain anionic phospholipids such as phosphatidylinositol (PI) (21). MBP is believed to activate the classical pathway of complement through the interaction and activation of the C1r2C1s2 complex (1). Furthermore, MBP itself may act as an opsonin having an affinity for the macrophage C1q receptor (e.g., as expressed by human Kupffer cells) (22). In contrast to charged liposomes, neutral egg phosphatidylcholine (PC) or lysoPC vesicles activate complement only after prolonged incubation in serum, presumably via CRP (23). Recently, Nilsson et al. (24) demonstrated a unique set of conformational changes related to a target adsorbed form of C3. This was evident from the exposure of neo-antigenic epitopes detected by mono- and polyclonal antibodies specific for bound C3 fragments. A subset of epitope, which occurs during denaturation and binding of C3, is capable of forming an initiating C3 convertase in the presence of factors B and D, leading to initiation of complement activation via the alternative pathway. It is therefore possible that nonspecific adsorption of human C3 on to the liposome surface in general could lead to complement activation via the alternative pathway.

Incorporation of cholesterol into the liposomal bilayer influences complement activation in a dose-dependent manner; increasing the cholesterol content of the vesicles increases the extent of complement activation (5,20). Here, facilitation of complement activation may be due to the formation of crystalline cholesterol (a potent activator of complement) and/or changes in bilayer structure, fluidity, and surface curvature, thus affecting lipid-protein interaction (e.g., C1q-phospholipid). Vesicle size also plays a critical role in complement activation (18,20,25). From static *in vitro* studies it appears that at a fixed lipid concentration, larger liposomes (200 nm and above) are more efficient at activating complement than are smaller vesicles. This probably suggest the importance of geometric factors and surface dynamics on the initial assembly of proteins involved in complement activation.

Immunoblotting studies have shown that the exposure of complement-activating vesicles to serum is associated with deposition of degradative C3 components and C9 (5,15,20,26). However, analysis of the pattern of C3 degradation products and their mode of linkage to the surface of liposomal formulations needs to be studied in detail. Such experiments may explain why a particular type of complement-activating vesicle exhibits different pharmacokinetics and clearance behavior from the blood when compared to another. Nevertheless, the deposition of C3 degradative products onto the surface of liposomes could stimulate vesicle recognition by activated complement receptors of polymorphonuclear cells (PMN), Kupffer cells, and spleen macrophage in rats, monkeys, and humans. However, murine Kupffer cells apparently lack complement receptors for C3 fragments, as they are hardly detectable by immunohistochemical techniques (11). The inability to detect these fragments could be due to the absence of such receptors or to blockade by bound ligands. Experiments with cDNA probes by *in situ* hybridization are necessary to determine whether the mRNA for C3 receptors is present in murine Kupffer cells. Nevertheless, this apparent lack of C3 receptors on mouse Kupffer cells suggests the involvement of other mechanisms for liposome uptake. Macrophage clearance and recognition of liposomes in knockout mice with abnormalities in the complement component C3 could further clarify this issue (27). Erythrocytes as well as platelets also express complement receptors and therefore are likely to participate in liposome clearance. For instance, Loughrey et al. (28) demonstrated C3b-mediated interaction of liposomes with rat platelets and suggested that such an interaction will result in removal of liposomes by the reticuloendothelial system (RES) due to formation of platelet-liposome microaggregates. Even though human platelets lack CR1, the receptor is expressed by blood monocytes, PMN, and erythrocytes (11). The role of these cells in liposome clearance has been neglected in the past. Since human and primate erythrocytes can bind to immune complexes (via a C3b-CR1 interaction) and transfer them to phagocytic cells during erythrocyte passage through the liver and spleen, a role for erythrocytes in liposome transfer to tissue macrophages seems possible. This speculation is worthy of investigation, because circulating erythrocytes outnumber circulating leukocytes and the vast majority of CR1 receptors present in circulation are located on the erythrocytes.

The association of C9 with liposomal membranes could be indicative of the activation of the entire complement pathway and possibly the assembly of membrane attack complexes (MACs) on the vesicle bilayer. The pores induced by MAC could also serve as sites for insertion of other plasma proteins into lipid bilayers. This mechanism may be responsible for murine Kupffer cell recognition of liposome following complement activation. Finally, activation of complement by liposomes *in vivo* may have effects beyond opsonization or MAC formation. For example, pigs have natural antibodies to cholesterol and phospholipids, and infusion of certain liposome compositions into these animals causes anaphylaxis or death (14).

Apolipoproteins

Association of plasma apolipoproteins with liposomes has been reported (see reference 29 for a recent review). Such associations may bear some importance for liposome recognition and clearance from the blood by both phagocytic and nonphagocytic cells. Experiments with transgenic animals that overexpress or lack several apolipoproteins (A-I, A-II, A-IV, C, E, B) or their corresponding receptors (30) (see also Scavenger Receptors section) are beginning to clarify this matter. For example, studies in apo E-deficient mice demonstrated a role for apo E in recognition and uptake of cholesterol-containing, small-sized egg PC liposomes by hepatocytes, presumably via low-density lipoprotein receptors (31). PS-containing liposomes adsorb significantly more apo E than do neutral liposomes, but their sequestration by the liver of apo E-deficient mice was found to be higher than that in control animals. Although Kupffer cells were predominant in liposome uptake, a significant fraction of PS-vesicles were cleared by hepatocytes. Therefore, recognition of PS-vesicles by hepatocytes is either mediated by apolipoproteins other than apo E or is independent of the opsonization processes (see also Scavenger Receptors section). In contrast to hepatocytes, the uptake of both neutral and PS-containing liposomes by splenic macrophages of apo E-deficient knock-out mutant mice is independent of apo E (31).

Recently, it was suggested that autoantibodies to phospholipids bind to oxidized anionic lipids (e.g., cardiolipin), but not to a reduced form of an anionic lipid that cannot undergo oxidation (9). The neoepitopes recognized by some auto antiphospholipid antibodies consist of adducts formed between the breakdown products of oxidized phospholipids (e.g., aldehydes generated during the decomposition of oxidized polyunsaturated fatty acids) and associated proteins, particularly the "reverse" acute-phase apolipoprotein H (β2-glycoprotein I) (9). Therefore, apolipoprotein H may mediate the phagocytic uptake of anionic vesicles either directly via the corresponding receptors on macrophages or in association with other blood proteins, such as anti-phospholipid antibodies (i.e, via complement and/or Fc receptors). Indeed, a recent study in mice demonstrated the association of apolipoprotein H with rapidly cleared anionic vesicles injected at low doses (32). Interestingly, apolipoprotein J, which shares some structural similarities with apolipoprotein H, has no affinity for anionic vesicles (32).

A Putative Egg PC/Cholesterol Opsonin

In rats, large size cholesterol-containing liposomes (700–800 nm) are cleared rapidly from the blood by the liver.

Complement activation was suggested to be a key mechanism for the hepatic clearance of such vesicles (33,34). On the other hand, numerous *in vivo* studies in rats have demonstrated that smaller sized cholestrol-containing vesicles (100–400 nm) localize less efficiently to the liver; spleen and bone marrow tend to play a major role in the clearance of such liposomes from the blood (reviewed in reference 35). Studies with freshly isolated rat Kupffer cells demonstrated a minor role for rat serum complement in recognition of both cholesterol-poor (20 mole% cholesterol content) and cholesterol-rich (46.6 mole% cholesterol) egg PC vesicles of 100–400 nm (despite the complement-activating nature of such vesicles and the presence of active complement receptors on isolated Kupffer cells) (11,36). However, serum displayed a dual role in Kupffer cell recognition of liposomes: It enhanced the uptake of cholesterol-poor vesicles but suppressed that of cholesterol-rich counterparts. The enhanced uptake of cholesterol-poor vesicles was attributed to the presence of an unidentified heat-stable calcium-sensitive serum protein (37,38). Two heat-stable rat serum proteins, responsible for suppression of liposome uptake by Kupffer cells, were also partially purified (11,35). A tentative hypothesis (11) has suggested that a balance between the blood opsonic molecule and these suppressive proteins (dysopsonins) could regulate the quantity and the rate of clearance of liposomes from the blood by Kupffer cells. Dysopsonins could modulate the rate of liposome uptake by reducing the amount of liposome-bound opsonin and hence protect Kupffer cells from being destroyed by excessive binding and ingestion of liposomes, particularly for those vesicles that are more resistant toward lysosomal esterases (39). This attractive speculation is rather analogous to nonmacrophage Hepatoma G2 cells, where the uptake of egg PC liposomes is mediated by apo E (40). Although apolipoproteins A-IV and A-I become associated with liposomes, these lipoproteins modulate the receptor-mediated uptake of vesicles by reducing the amount of liposome-bound apo E.

In contrast to hepatic macrophages, from *in vitro* cell studies it appears that heat-labile serum factors (e.g., some components of the complement system) play an important role on the uptake of cholesterol-rich liposomes by rat spleen and bone marrow phagocytic cells (reviewed in reference 11). These simple *in vitro* studies are in agreement with *in vivo* tissue distribution of intravenously injected cholesterol-containing vesicles and indicate the involvement of different serum factors on liposome recognition by different phagocytic cells.

Fetuin (Human α2-HS-Glycoprotein)

Fetuin is an acidic negative acute-phase glycoprotein with three N-linked and three O-linked oligosaccharide chains, the terminal sugar residues of which are rich in sialic acid. Hepatocytes are the principal cell source of circulating fetuin. The biological role of fetuin is unknown, although it has been implicated as an immunomodulator that can participate in the stimulation of bacterial phagocytosis by neutrophils and promotion of endocytosis by mouse macrophages (41). Recently, fetuin was shown to act as an opsonin for cationic molecules (e.g., spermine) (41). Macrophages use fetuin to assess the abundance of extracellular spermine, which, in turn, down regulates synthesis of proinflammatory cytokines and prevents excessive inflammation (41). A role for fetuin in stability and blood clearance of cationic liposomes is worthy of investigation.

von Willebrand Factor (and Thrombospondin)

von Willebrand factor is an adhesive protein that mainly contributes to hemostatic plug formation, and its function is limited to the maintenance of intact blood vessels. This protein is stored in the α-granules of platelets or in endothelial cell-specific Weibel-Palade bodies. Although the contribution of von Willebrand factor in immune defense has not been recognized fully, a recent study demonstrated that plasma von Willebrand factor, as well as thrombospondin, opsonized sulfatide-rich particles and enhanced their phagocytosis by human monocytes independent of immunoglobulins and complement (42). Thrombospondin had three times more affinity than von Willebrand factor for sulfatide-rich particles. It is reasonable to speculate that these macromolecules may opsonize sulfatide-rich or other anionic vesicles and promote their recognition by the scavengers of the RES, either directly (presumably via the scavenger receptor CD36 and the integrin $\alpha_v\beta_3$) or via transfer by platelets (e.g., following interaction with platelet CD36 or the integrin $\alpha_{IIb}\beta_3$) (43).

OPSONIC-INDEPENDENT RECOGNITION

Recently, Liu and Liu (44) demonstrated that the uptake of liposomes by perfused mouse liver, when expressed as percentage of added dose, is insensitive to the presence of serum. This led to the suggestion that the uptake was directly related to surface characteristics of the vesicles and direct recognition by macrophage receptors. For example, while the uptake of neutral egg PC/cholesterol vesicles was relatively poor, inclusion of low concentrations of either DCP or PS in the lipid bilayer dramatically enhanced vesicle uptake by the perfused liver both in the absence and the presence of serum. Furthermore, preperfusion of liver with neutral or DCP-containing vesicles had no effect on the uptake of PS liposomes. This further suggested the existence of multiple serum-independent mechanisms of liposome recognition (44,45). Similarly, other workers (46) have also suggested that the liposome uptake by certain cell lines is also insensitive to the presence of serum. For instance, endocytosis of liposomes containing 9 mole% of various negatively charged lipids by CV1 cells, an African green monkey kidney cell line, as well as J774 cells, a murine macrophage-like cell line, was insensitive to the presence of serum (46). The same was true with liposomes bearing higher percentages of negative charge in the case of CV1 cells. However, uptake by J774 cells was strongly reduced by serum, particularly at high surface-charge density. Furthermore, liposome uptake by J774 cells displayed a strong inhibitory response toward polyanions, whereas uptake by CV1 cells was insensitive to such agents (46). Although the views of Liu and Liu (44) and others (45,46) seem logical with respect to the existence of a serum-independent mechanism in liposome recognition by macrophages, a serum-dependent mechanism can also explain these observations. Let us assume that two macrophage receptors R1 and R2 participate in liposome recognition; R1 recognizes a particular phospholipid headgroup (a serum-independent mechanism) while R2 binds to and internalizes protein-coated vesicles (serum-dependent uptake). However, when uptake is

expressed as a percentage of the added dose, similar values are obtained. This raises doubt as to which mechanism is the true representation of liposome uptake *in vivo*. This problem can be resolved by identifying and cloning the participant receptors and performing liposome distribution studies in transgenic animals.

If macrophages, hepatocytes and liver endothial cells can recognize phospholipid headgroups *in vivo,* then what are the likely receptors that participate in this process and initiate vesicle internalization?

Hepatic Lectin Receptors

Recent *in vitro* studies have demonstrated that the rat liver MBP binds strongly to PG liposomes and to some extent to PS liposomes (21). Competition studies further indicated that the binding sites for PG and PS in the liver-MBP molecule might be different from each other (21). Although these observations provide evidence for a novel type of ligand binding specificity for liver-MBP, the physiological significance of liver-MBP binding to acidic phospholipids remains to be clarified.

Scavenger Receptors (SRs)

SRs are well known to endocytose chemically modified lipoproteins (47). Other ligands for SRs include polyanionic macromolecules, bacterial polysaccharides, silica, and possibly anionic liposomes. Six classes of SRs have now been recognized on the basis of structural homologies and ligand-binding properties (reviewed in references 48 and 49). All are plasma membrane proteins that are expressed on a number of cell types; in particular, cells of the immune system and endothelia. With regard to liposome recognition, macrophage classes A, B, and D SRs are of potential interest. In addition, endothelial and hepatocyte SRs may also participate in liposome clearance from the blood.

Class A SRs

Class A SRs are essentially restricted in expression to macrophages. Class A receptors are trimeric: three members have been identified (two type A receptors and MARCO) (48). SR-AI and SR-AII are alternative transcripts of the same gene. The type II receptor is identical to type I, except that the cysteine-rich domain is replaced by a six-residue carboxyl-terminus. Both SR-AI and SR-AII have four identified domains: a cytoplasmic tail, a transmembrane domain, an alpha-helical coil, and a collagenous domain. The binding site for acetylated low-density lipoprotein, and possibly microbial ligands, is located in the collagenous domain (49). It is also interesting to note that C1q also has a collagenous domain that exhibits similar ligand-binding properties to SR-AI (e.g., binding to polyanions and anionic liposomes, as discussed earlier), but C1q is not able to bind acetylated low-density lipoproteins (50). The third member, MARCO, has cytoplasmic, transmembrane, and spacer domains that are not related to SR-AI and SR-AII, but a collagenous and a cysteine-rich domain that are homologous to SR-AI (49). This receptor was shown to recognize intact Gram-negative bacteria (51).

There have been contrasting views regarding a role for class A SRs in liposome recognition. Early studies of Dijkstra et al. (52) excluded the participation of such receptors on the uptake of liposomes containing 10 mole% PS by isolated rat Kupffer cells in culture. However, the binding of PS-containing liposomes to Kupffer cells was competitively inhibited by carboxylated latex beads, emphasizing the importance of multiple negatively charged groups for macrophage recognition. Recently, a role for class A SRs in the uptake of liposomes containing 30 mole% or more PS by cultured rat Kupffer cells was suggested (31,53). Therefore, it is very likely that particular stereo organization and density of charge are essential for liposome recognition by Kupffer cell SRs *in vitro*. Surprisingly, preinjection of rats with polyinosinic acid (an inhibitor of class A SRs) failed to inhibit the uptake of such PS-containing vesicles by Kupffer cells (53). This probably indicates the importance of other pathways or macrophage SRs, such as CD36 and macrosialin (see below), for *in vivo* liposome recognition. Confusion also exists with regard to liposome recognition by peritoneal macrophage SRs. Competition studies with various ligands for the class A SRs showed that acetylated low-density lipoprotein, dextran sulfate, or fucoidan was able to compete for up to 60% of the binding of PS-containing liposomes to mouse peritoneal macrophages (54). Furthermore, PS-containing vesicles were able to compete for more than 90% of the binding of acetylated low-density lipoproteins to mouse peritoneal macrophages. While these studies indicated a role for class A SRs, COS cells that were transfected with type AI and type AII receptors were not able to bind and internalize PS-incorporated vesicles (54). Recently, mice deficient in type I and type II macrophage SR-A were generated by disrupting exon 4 of the macrophage SR-A gene (55). Biodistribution of liposomes in such transgenic animals could clarify the role of macrophage SR-A in recognition of anionic vesicles. Similarly, it would be interesting to study the response of mice with a targeted disruption of the MARCO gene to a range of anionic phospholipid vesicles.

Class B SRs (CD36, BI, and BII)

These receptors are expressed on macrophages, platelets, adipocytes, and some endothelial cells, and all have a single extracellular domain with a carboxyl-terminal region that has conserved cysteine residues (48). So far, the identified ligands for class B receptors include thrombospondin, collagen, oxidized LDL, PS, and PS-incorporated liposomes (56–58). Gene transfer of CD36 to nonphagocytic cells also confers recognition for PS (58).

Rat liver hepatocytes express a BI type SR that participates on the uptake of high-density lipoprotein cholesteryl esters (HDL-CE) without parallel apolipoprotein uptake (59). Anionic phospholipid liposomes can inhibit HDL-CE uptake by 40%, whereas neutral vesicles are ineffective (59). Therefore, a role for rat hepatocyte SR-BI in recognition of anionic vesicles cannot be ruled out.

Class D SRs: Murine and Rat Macrophage Macrosialin (Human Homologue CD68)

Macrosialin or CD68 are macrophage-specific members of a family of widely distributed and extensively O- and N-glycosylated lysosome-associated membrane glycoproteins with a unique mucin-like extracellular domain (47). Macrosialin is overexpressed in thioglycollate-elicited murine macrophages (60). Treatment of murine macrophages with phor-

bol ester also increases the expression of macrosialin (60). The expression of macrosialin by rat Kupffer cells has been demonstrated recently (61). Class D receptors are capable of recognizing both oxidized low-density lipoproteins as well as PS-rich liposomes (47,60). Although the predominant intracellular localization of macrosialin in late endosomes contradicts its possible role as an anionic phospholipid receptor, it is possible that macrosialin exhibits very rapid translocation between intracellular sites and the plasma membrane. A definitive demonstration that macrosialin or CD68 is an important player in liposome recognition is dependent on future analysis of macrosialin knockout animals.

Other Endothelial SRs

In addition to class B receptors, endothelial cells express a number of lectin-like SRs (e.g., classes E and F) that participate in the uptake of low-density lipoproteins, apoptotic cells, and possibly anionic liposomes (62–65). To date, limited studies have shown that, in a serum-free media, rat liver endothelial cells are able to recognize liposomes containing 30 mole% PS via the SRs (53). However, in the presence of serum the uptake was inhibited, indicating that the adsorption of serum proteins onto the liposome surface can mask the PS headgroups and hence block interaction with SRs. Similarly, vesicles containing 100% PS also failed to interact with liver endothelial cells *in vivo* (53). Studies with annexin-V are still necessary to demonstrate whether serum protein can mask PS headgroups in vesicles of different surface curvature; indeed, annexin-V has high affinity for apoptotic cells or activated platelets with exposed PS *in vivo* (66). In contrast to the above-described liposome formulations, rat liver endothelial cells recognized and cleared intravenously injected polyanionized proteoliposomes (e.g., polyaconitylated human serum albumin-grafted liposomes) of 100 nm in diameter via SRs (67). Presumably, the projected polyaconitylated albumin molecules can expose the correct conformational requirements of the negative charge cluster for recognition by such receptors. However, the targeting was not fully specific, because Kupffer cells and to some extent hepatocytes participated in the clearance process. Increasing the size of the vesicles also resulted in efficient capture by Kupffer cells (67). Nevertheless, it seems that the abundant presence of various types of SRs in the liver plays an important role in intrahepatic distribution of anionic vesicles.

Macrophage FcγRII-B2

The murine 50 kDa FcγRII-B2 macrophage protein has also been identified as a putative oxidized low-density lipoprotein receptor that mediates internalization independent of IgG (68). Preliminary results also indicate that FcγRI also binds oxidized low-density lipoprotein with high affinity (69). An immunoglobulin-independent recognition mechanism of anionic vesicles by such classes of Fc receptors seems possible.

CD14 Antigen

This molecule is a physiologically important receptor for lipopolysaccharide (which is also a ligand for SR-AI) and is expressed strongly by blood monocytes and activated granulocytes (70). Recently, CD14 was shown to bind to apoptotic B cells, presumably recognizing the exposed PS (70). In the human liver, the expression of CD14 on Kupffer cells is negligible (12). However, in both acute and chronic liver diseases, most liver macrophages are positive for CD14 (12). It is possible that liver macrophages in various liver diseases are activated to express CD14. Therefore, a role for CD14 in the recognition of modified lipoproteins as well as anionic liposomes by monocytes and stimulated liver macrophages must be considered.

A Putative Stearylamine Receptor

Recently, it was demonstrated that treatment of rats with diethylstilbestrol (a synthetic estrogen) results in overexpression or increased activity of a putative plasma membrane receptor in Kupffer cells (or newly recruited liver macrophages) that can recognize the surface determinants of stearylamine incorporated egg PC vesicles in the blood (71). *In vitro* competition studies further demonstrated that this putative receptor plays a minor role in the clearance of neutral and anionic vesicles (e.g., DCP-incorporated vesicles).

WHY SO MANY MECHANISMS?

It appears that phagocyte recognition of phospholipid vesicles is a complex phenomenon and it is unlikely that this complexity is merely a reflection of *in vitro* approaches. Liposomes represent a simple model for biological membranes and it is therefore not surprising to see that they share similar mechanisms, for example, with apoptotic or damaged cells for recognition by phagocytes. Not all macrophages are identical: considerable heterogeneity with respect to phenotype and physiological properties exists between different types of macrophages and even among macrophages of the same tissue (11). Therefore, particular populations of phagocytes may employ one predominant recognition mechanism.

There are also data to show that phagocyte receptors may need to cooperate to achieve phagocytosis of certain particulates and cells. Therefore, cooperation between fibronectin (or immunoglobulins) with complement (72) or $\alpha_v\beta_3$ with CD36 (43) may increase the efficiency of liposome phagocytosis and clearance from the blood. The presence of multiple mechanisms may further suggest an arrangement based on a recognition hierarchy. For example, a particular macrophage receptor might recognize the earliest changes associated with the liposome surface in the blood, while other receptors might recognize liposomes at a later stage, thus ensuring complete removal of vesicles from the circulation.

Finally, the advances in immunological sciences and the use of increasingly sophisticated genetic approaches to modify selected macrophage receptor activity or generating abnormalities in circulating levels of candidate opsonins will begin to define more clearly the *in vivo* contribution of each of the different receptors and opsonic molecules in macrophage recognition of phospholipid vesicles. Such biochemical and immunological approaches will facilitate and focus the mind of pharmaceutical scientists for rational design of liposomal-based medicines.

REFERENCES

1. S. C. Semple, A. Chonn, and P. R. Cullis. Interactions of liposomes and lipid-based carrier systems with blood proteins: Rela-

tion to clearance behaviour *in vivo*. *Adv. Drug Deliv. Rev.* **32**:3–17 (1998).
2. F. Bonté and R. L. Juliano. Interactions of liposomes with serum proteins. *Chem. Phys. Lipids* **40**:359–372 (1986).
3. S. M. Moghimi and H. M. Patel. Tissue specific opsonins for phagocytic cells and their different affinity for cholesterol-rich liposomes. *FEBS Lett.* **233**:143–147 (1988).
4. A. Chonn, S. C. Semple, and P. R. Cullis. Association of blood proteins with large unilamellar liposomes *in vivo*. Relation to circulation lifetimes. *J. Biol. Chem.* **267**:18759–18765 (1992).
5. A. Chonn, P. R. Cullis, and D. V. Devine. The role of surface charge in the activation of the classical and alternative pathways of complement by liposomes. *J. Immunol.* **146**:4234–4241 (1991).
6. E. T. M. Dams, P. Laverman, W. J. G. Oyen, G. Storm, G. L. Scherphof, J. W. M. Van Der Meer, F. H. Corstens, and O. C. Boerman. Accelerated blood clearance and altered biodistribution of repeated injections of sterically stabilized liposomes. *J. Pharmacol. Exp. Ther.* **292**:1071–1079 (2000).
7. H. M. Patel. Serum opsonins and liposomes: Their interaction and opsonophagocytosis. *Crit. Rev. Ther. Drug Carrier Syst.* **9**: 39–90 (1992).
8. C. R. Alving and G. M. Swartz, Jr. Antibodies to cholesterol, cholesterol conjugates and liposomes: Implications for atherosclerosis and autoimmunity. *CRC Crit. Rev. Immunol.* **10**:441–453 (1991).
9. S. Hörkkö, E. Miller, D. Ware Branch, W. Palinski, and J. L. Witztum. The epitopes for some antiphospholipid antibodies are adducts of oxidized phospholipid and β2 glycoprotein 1 (and other proteins). *Proc. Natl. Acad. Sci. USA* **94**:10356–10361 (1997).
10. S. M. Moghimi and S. S. Davis. Innovations in avoiding particles clearance from blood by Kupffer cells: Cause for reflection. *Crit. Rev. Ther. Drug Carrier Syst.* **11**:31–59 (1994).
11. S. M. Moghimi and H. M. Patel. Serum-mediated recognition of liposomes by phagocytic cells of the reticuloendothelial system—The concept of tissue specificity. *Adv. Drug Deliv. Rev.* **32**:45–60 (1998).
12. M. Tomita, K. Yamamoto, H. Kobashi, M. Ohmoto, and T. Tsuji. Immunohistochemical phenotyping of liver macrophages in normal and diseased human liver. *Hepatology* **20**:317–325 (1994).
13. D. V. Devine and J. M. J. Marjan. The role of immunoproteins in the survival of liposomes in the circulation. *Crit. Rev. Ther. Drug Carr. Syst.* **14**:105–131 (1997).
14. J. Szebeni. The interaction of liposomes with the complement system. *Crit. Rev. Ther. Drug Carr. Syst.* **15**:57–89 (1998).
15. K. Funato, R. Yoda, and H. Kiwada. Contribution of complement system on destabilization of liposomes composed of hydrogenated egg phosphatidylcholine in fresh rat plasma. *Biochim. Biophys. Acta* **1103**:198–204 (1992).
16. J. Marjan, Z. Xie, and D. V. Devine. Liposome-induced activation of the classical complement pathway does not require immunoglobulin. *Biochim. Biophys. Acta* **1192**:35–44 (1994).
17. A. J. Bradley, E. Maurer Spurej, D. E. Brooks, and D. V. Devine. Unusual electrostatic effects on binding of C1q to anionic liposomes: Role of anionic phospholipid domains and their line tension. *Biochemistry* **38**:8112–8123 (1999).
18. A. J. Bradley, D. V. Devine, S. M. Ansell, J. Janzen, and D. E. Brooks. Inhibition of liposome-induced complement activation by incorporated poly(ethylene glycol)-lipids. *Arch. Biochem. Biophys.* **357**:185–194 (1998).
19. R. L. Richards, H. Gewurz, A. P. Osmand, and C. R. Alving. Interactions of C-reactive protein and complement with liposomes. *Proc. Natl. Acad. Sci. USA* **74**:5672–5680 (1977).
20. D. V. Devine, K. Wong, K. Serrano, A. Chonn, and P. R. Cullis. Liposome-complement interactions in rat serum: Implications for liposome survival studies. *Biochim. Biophys. Acta* **1191**:43–51 (1994).
21. Y. Kuroki, T. Honma, H. Chiba, H. Sano, M. Saitoh, Y. Ogasawara, H. Sohma, and T. Akino. A novel type of binding specificity to phospholipids of rat mannose-binding proteins isolated from serum and liver. *FEBS Lett.* **414**:387–392 (1997).
22. M. Kuhlman, K. Joiner, and R. A. Ezekowitz. The human mannose-binding protein functions as an opsonin. *J. Exp. Med.* **169**: 1733–1741 (1989).
23. J. E. Volanakis, and K. W. A. Wirtz. Interaction of C-reactive protein with artificial phosphatidylcholine bilayers. *Nature* **281**: 155–157 (1979).
24. U. R. Nilsson, K. E. Storm, H. Elwing, and B. Nilsson. Conformational epitopes of C3 reflecting its mode of binding to an artificial polymer surface. *Mol. Immunol.* **30**:211–219 (1993).
25. H. Harashima, K. Sakata, K. Funato, and H. Kiwada. Enhanced hepatic uptake of liposomes through complement activation depending on the size of liposomes. *Pharm. Res.* **11**:402–406 (1994).
26. H. Harashima, M. Matsuo, and H. Kiwada. Identification of proteins mediating clearance of liposomes using a liver perfusion system. *Adv. Drug Deliv. Rev.* **32**:61–79 (1998).
27. S. R. Barnum, J. L. Jones, U. Mullerladner, A. Samimi, and I. L. Campbell. Chronic complement C3 gene expression in the CNS of transgeneic mice with astrocyte-targeted interleukin-6 expression. *Glia* **18**:107–117 (1996).
28. H. C. Loughrey, M. B. Bally, L. W. Reinish, and P. R. Cullis. The binding of phosphatidylglycerol liposomes to rat platelets is mediated by complement. *Throm. Haemost.* **64**:172–176 (1990).
29. W. V. Rodrigueza, M. C. Phillips, and K. J. Williams. Structural and metabolic consequences of liposome-lipoprotein interactions. *Adv. Drug Deliv. Rev.* **32**:31–43 (1998).
30. J. L. Breslow. Mouse models of atherosclerosis. *Science* **272**:685–688 (1996).
31. G. L. Scherphof, and J. A. A. M. Kamps. Receptor versus nonreceptor mediated clearance of liposomes. *Adv. Drug Deliv. Rev.* **32**:81–97 (1998).
32. A. Chonn, S. C. Semple, and P. R. Cullis. β2-Glycoprotein I is a major protein associated with very rapidly cleared liposomes *in vivo,* suggesting a significant role in the immune clearance of 'non-self' particles. *J. Biol. Chem.* **270**:25845–25849 (1995).
33. T. Ishida, K. Funato, S. Kojima, R. Yoda, and H. Kiwada. Enhancing effect of cholesterol on the elimination of liposomes from circulation is mediated by complement activation. *Int. J. Pharmaceut.* **156**:27–37 (1997).
34. T. M. Huong, H. Harashima, and H. Kiwada. *In vivo* studies on the role of complement in the clearance of liposomes in rats and guinea pigs. *Biol. Pharm. Bull.* **22**:515–520 (1999).
35. S. M. Moghimi. Opsono-recognition of liposomes by tissue macrophages. *Int. J. Pharmaceut.* **162**:11–18 (1998).
36. S. M. Moghimi and H. M. Patel. Differential properties of organ-specific serum opsonins for liver and spleen macrophages. *Biochim. Biophys. Acta* **984**:379–383 (1989).
37. S. M. Moghimi and H. M. Patel. Calcium as a possible modulator of Kupffer cell phagocytic function by regulating liver-specific opsonic activity. *Biochim. Biophys. Acta* **1028**:304–308 (1990).
38. S. M. Moghimi and H. M. Patel. Altered tissue-specific opsonic activities and opsono-recognition of liposomes in tumour-bearing rats. *Biochim. Biophys. Acta* **1285**:56–64 (1996).
39. J. T. P. Derksen, J. D. Baldeschwieler, and G. L. Scherphof. *In vivo* stability of ester- and ether-linked phospholipid-containing liposomes as measured by perturbed angular correlation spectroscopy. *Proc. Natl. Acad. Sci. USA* **85**:9768–9772 (1988).
40. C. L. Bisgaier, M. V. Siebenkas, and K. J. Williams. Effects of apolipoproteins A-IV and A-I on the uptake of phospholipid liposomes by hepatocytes. *J. Biol. Chem.* **264**:862–866 (1989).
41. H. Wang, M. Zhang, M. Bianchi, B. Sherry, A. Sama, and K. J. Tracey. Fetuin (α_2-HS-glycoprotein) opsonizes cationic macrophage-deactivating molecules. *Proc. Natl. Acad. Sci. USA* **95**: 14429–14434 (1998).
42. M. V. Serra, F. Mannu, A. Matera, F. Turrini, and P. Arese. Enhanced IgG- and complement-independent phagocytosis of sulfatide-enriched human erythrocytes by human monocytes. *FEBS Lett.* **311**:67–70 (1992).
43. J. S. Savill, P. M. Henson, and C. Haslett. Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. *J. Clin. Invest.* **90**:1513–1522 (1992).
44. F. Liu and D. Liu. Serum independent liposome uptake by mouse liver. *Biochim. Biophys. Acta* **1278**:5–11 (1996).
45. Q. Hu and D. Liu. Co-existance of serum-dependent and serum-independent mechanism for liposome clearance and involvement of non-Kupffer cells in liposome uptake by mouse liver. *Biochim. Biophys. Acta* **1284**:153–161 (1996).
46. K. D. Lee, K. Hong, and D. Papahadjopoulos. Recognition of liposomes by cell: *in vitro* binding and endocytosis mediated by

specific lipid head groups and surface charge density. *Biochim. Biophys. Acta* **1103**:185–197 (1992).
47. R. P. da Silva, N. Platt, W. J. S. de Villiers, and S. Gordon. Membrane molecules and macrophage endocytosis: scavenger receptor and macrosialin as markers of plasma-membrane and vacuolar functions. *Biochem. Soc. Trans.* **24**:220–224 (1996).
48. D. R. Greaves, P. J. Gough, and S. Gordon. Recent progress on defining the role of scavenger receptors in lipid transport, atherosclerosis and host defence. *Curr. Opin. Lipidol.* **9**:425–432 (1998).
49. T. Kodama, T. Doi, H. Suzuki, K. Takahashi, Y. Wada, and S. Gordon. Collagenous macrophage scavenger receptors. *Curr. Opin. Lipidol.* **7**:287–291 (1996).
50. S. Acton, D. Resnick, M. Freeman, Y. Ekkel, J. Askenas, and M. Krieger. The collagenous domains of macrophage scavenger receptors and complement component C1q mediate similar, but not identical, binding specificities for polyanionic ligands. *J. Biol. Chem.* **268**:3530–3537 (1993).
51. O. Elomaa, M. Kangas, C. Sahlberg, J. Tuukkanen, R. Sormunen, A. Liakka, I. Thesleff, G. Kraal, and K. Tryggvason. Cloning of a novel bacteria-binding receptor structurally related to scavenger receptors and expressed in a subset of macrophages. *Cell* **80**:603–609 (1995).
52. J. Dijkstra, M. Van Galen, and G. Scherphof. Influence of liposome charge on the association of liposomes with Kupffer cells *in vitro*. Effects of divalent cations and competition with latex particles. *Biochim. Biophys. Acta* **813**:287–297 (1985).
53. J. A. A. M. Kamps, H. W. M. Morselt, and G. L. Scherphof. Uptake of liposomes containing phosphatidylserine by liver cells *in vivo* and by sinusoidal liver cells in primary culture: *In vivo-in vitro* differences. *Biochem. Biophys. Res. Commun.* **256**:57–62 (1999).
54. K. Nishikawa, H. Arai, and K. Inoue. Scavenger receptor-mediated uptake and metabolism of lipid vesicles containing acidic phospholipids by mouse peritoneal macrophages. *J. Biol. Chem.* **265**:5226–5231 (1990).
55. H. Suzuki, Y. Kurihara, M. Takeya, N. Kamada, M. Kataoka, K. Jishage, O. Ueda, H. Sakaguchi, T. Higashi, T. Suzuki, Y. Takashima, Y. Kawabe, O. Cynshi, Y. Wada, M. Hondo, H. Kurihara, H. Aburatani, T. Doi, A. Matsumoto, S. Azuma, T. Noda, Y. Toyoda, H. Itakura, Y. Yazaki, S. Horiuchi, K. Takahashi, J. Kar Kruijt, T. J. C. van Berkel, R. P. Steinbrecher, S. Ishibashi, N. Maeda, S. Gordon, and T. Kodama. A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection. *Nature* **386**:292–296 (1997).
56. V. A. Fadok, D. R. Voelker, P. A. Campbell, J. J. Cohen, D. L. Bratton, and P. M. Henson. Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J. Immunol.* **148**:2207–2216 (1992).
57. G. Endemann, L. W. Stanton, K. S. Madden, C. M. Bryant, R. Tyler White, and A. A. Protter. CD36 is a receptor for oxidized low density lipoprotein. *J. Biol. Chem.* **268**:11811–11816 (1993).
58. M. Fukasawa, H. Adachi, K. Hirota, M. Tsujimoto, H. Arai, and K. Inoue. SRB1, a class B scavenger receptor, recognizes both negatively charged liposomes and apoptotic cells. *Exp. Cell Res.* **222**:246–250 (1996).
59. K. Fluiter and T. J. C. van Berkel. Scavenger receptor B1 (SR-B1) substrates inhibit the selective uptake of high-density-lipoprotein cholesteryl esters by rat parenchymal liver cells. *Biochem. J.* **326**:515–519 (1997).
60. M. Lougheed, C. M. Lum, W. H. Ling, H. Suzuki, T. Kodama, and U. Steinbrecher. High affinity saturable uptake of oxidized low density lipoprotein by macrophages from mice lacking the scavenger class A type I/II. *J. Biol. Chem.* **272**:12938–12944 (1997).
61. A. G. van Velzen, R. P. Da Silva, S. Gordon, and T. J. C. van Berkel. Characterization of a receptor for oxidized low-density lipoproteins on rat Kupffer cells: Similarity to macrosialin. *Biochem. J.* **322**:411–415 (1997).
62. T. Sawamura, N. Kume, T. Aoyama, H. Moriwaki, H. Hoshikawa, Y. Aiba, T. Tanaka, S. Miwa, Y. Katsura, T. Kita, and T. Masaki. An endothelial receptor for oxidized low-density lipoprotein. *Nature* **386**:73–77 (1977).
63. H. Adachi, M. Tsujimoto, H. Arai, and K. Inoue. Expression cloning of a novel scavenger receptor from human endothelial cells. *J. Biol. Chem.* **272**:31217–31220 (1998).
64. Y. B. de Rijke and T. J. C. van Berkel. Rat liver Kupffer and endothelial cells express different binding proteins for modified low density lipoproteins. *J. Biol. Chem.* **269**:824–827 (1994).
65. M. Nagase, S. Hirose, T. Sawamura, T. Masaki, and T. Fujita. Enhanced expression of endothelial oxidized low-density lipoprotein receptor (LOX-1) in hypertensive rats. *Biochem. Biophys. Res. Commun.* **237**:496–498 (1997).
66. F. G. Blankenberg, P. D. Katsikis, J. F. Tait, R. E. Davis, L. Naumovski, K. Ohtsuki, S. Kopiwoda, M. J. Abrams, M. Darkes, R. C. Robbins, H. T. Maecker, and H. W. Strauss. *In vivo* detection and imaging of phosphatidylserine expression during programmed cell death. *Proc. Natl. Acad. Sci. USA* **95**:6349–6354 (1998).
67. J. A. A. M. Kamps, H. W. M. Morselt, P. J. Swart, D. K. F. Meijer, and G. L. Scherphof. Massive targeting of liposomes, surface-modified with anionized albumins, to hepatic endothelial cells. *Proc. Natl. Acad. Sci. USA* **94**:11681–11685 (1997).
68. L. W. Stanton, R. T. White, C. M. Bryant, A. Protter, and G. Endemann. A macrophage Fc receptor for IgG is also a receptor for oxidized low-density-lipoprotein. *J. Biol. Chem.* **267**:22446–22451 (1992).
69. H. Arai, T. Kita, M. Yokode, S. Narumiya, and C. Kawai. Multiple receptors for modified low-density lipoproteins in mouse peritoneal-macrophages. Different uptake mechanisms for acetylated and oxidized low-density lipoproteins. *Biochem. Biophys. Res. Commun.* **159**:1375–1382 (1989).
70. A. Devitt, O. D. Moffatt, C. Raykundalia, J. D. Capra, D. L. Simmons, and C. D. Gregory. Human CD14 mediates recognition and phagocytosis of apoptotic cells. *Nature* **392**:505–509 (1998).
71. S. M. Moghimi. Hormonal-control of macrophage phagocytosis of phospholipid vesicles. *J. Liposome Res.* **10:**379–387 (2000).
72. J. F. Bohnsack, J. J. Oshea, T. Takahashi, and E. J. Brown. Fibronectin-enhanced phagocytosis of an alternative pathway activator by human culture-derived macrophages is mediated by the C4B/C3B complement receptor (CR-1). *J. Immunol.* **135**:2680–2686 (1985).