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Phagocytic uptake by mouse peritoneal macrophages of microspheres coated with phosphocholine or polyethylene glycol phosphate-derived perfluoroalkylated surfactants

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Abstract

A series of perfluoroalkylated amphiphiles derived from phosphocholine (PC) and polyethylene glycol (PEG) phosphates has been studied and compared to hydrocarbon analogs with respect to their ability to modify the in vitro protein adsorption, and phagocytic uptake by mouse peritoneal macrophages of polystyrene microspheres coated with these surfactants. A significant correlation between protein adsorption and phagocytosis was seen. Within the PC-derived amphiphiles investigated, those with the shorter C2 and C5 spacers between the fluorinated tail and the phosphocholine group, F8C2PC and F8C5PC, caused significantly lower protein adsorption and a decrease of phagocytic uptake of the microspheres in serum vs a buffer. Phagocytic uptake is then comparable to that observed when pegylated surfactants are used as the coating material. These effects were no longer seen when the spacer was longer, as in F8C11PC, with the non-fluorinated analogues C10PC and C15PC, or when one methyl group was replaced by an ethyl group in the phosphocholine polar head. The beneficial impact of the fluorinated tail thus appear to be related to its distance from the surfactant film's external surface and/or to the lipophobic character of the surfactant. Without serum present phagocytic uptake was lower for the fluorinated surfactants with hydrophilic PEG phosphate head-groups, F8C5PPEG 2000 and F8C5P[PEG 750]2, than for the PC derivatives, although it was significantly greater than with Pluronic F-68 or DSPE-PEG 5000. Phagocytosis was, however, not reduced in the presence of serum and the difference between these surfactants and the fluorinated PC derivatives was no longer appreciable.

Keywords: Phagocytosis; Peritoneal macrophage; Polystyrene microsphere; Perfluoroalkylated surfactant; Fluorinated amphiphile; Zeta potential; Serum protein adsorption; Hydrophilicity; Lipophobicity

1. Introduction

There is a paucity of highly effective surfactants that could be used in the preparation of emulsions, vesicles and other colloidal systems for biomedical applications. Numerous well-defined perfluoroalkylated surfactants have recently been synthesized which may fulfil this need (Riess, 1988; Riess et al., 1989; Greiner et al., 1993; Riess and Greiner, 1993; Riess and Krafft, 1994). Among the key objectives were the stabilization

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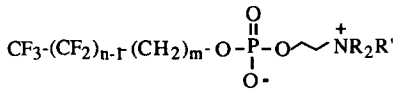
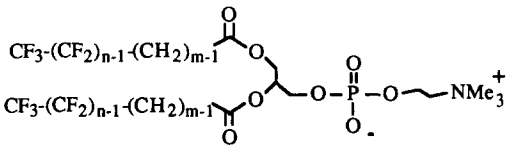
and control of the properties of injectable fluorocarbon emulsions destined to serve as oxygen carriers (blood substitutes), contrast agents for diagnosis and drug delivery (Riess, 1993, 1994a). It is likewise desirable to gain further mastery in liposome technology in order to facilitate the modulation of their characteristics and to extend their spectrum of applications (Riess, 1994a,b).

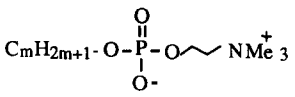
One of the serious challenges for all particle-based carriers, whether emulsion droplets, vesicles or capsules, destined to be injected in the circulation, is the control of their recognition and uptake by the mononuclear phagocytic system (MPS). Clearance of particles from the blood stream involves interaction with blood plasma components, resulting in opsonization followed by macrophage uptake, and/or in particle disintegration by lipid exchange and depletion (Scherphof et al., 1981; Gregoriadis, 1993). These phenomena were shown to depend on particle size (Okamoto et al., 1975; Kawaguchi and Koiwai, 1986) and on serum opsonic factors (Davis and Hansrani, 1985; Moghimi and Patel, 1988). Phagocytosis also strongly depends on the characteristics of the amphiphiles which compose the film which surrounds the particles and constitutes



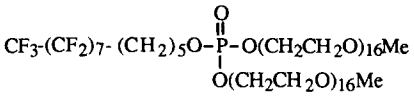
Fn	Cm	R	R'	Code name
n = 8	m = 2	Me	Me	F8C2PC
"	5	"	"	F8C5PC
"	11	"	"	F8C11PC
"	5	"	Et	F8C5PCEt



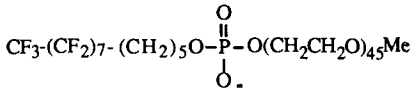
Fn	Cm	Code name
n = 6	m = 11	DF6C11PC
8	5	DF8C5PC



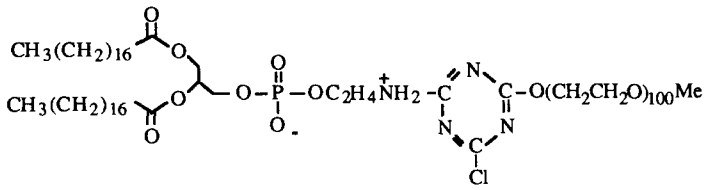
m	Code name
10	C10PC
15	C15PC



Code name : F8C5P[PEG750]2



Code name : F8C5PPEG2000



Code name : DSPE-PEG5000

Fig. 1. Molecular structure of the fluorinated single-chain phosphocholines, poly(ethylene glycol) phosphates and double-chain phosphatidylcholines, with their code names.

their external, exposed surface. Previous data indicate a reduction in particle uptake by avoiding net surface charge (Hsu and Juliano, 1982; Schwendener et al., 1984), increasing membrane rigidity (Juliano et al., 1985; Allen et al., 1989), surface hydrophilicity (Illum and Davis, 1984) and surface mobility (Blume and Cevc, 1993). The exact contribution of each one of these factors is, however, still a matter of debate. In the case of PEG-labelled 'stealth' liposomes, steric stabilization as well as surface mobility of the PEG chains is thought to be essential (Woodle and Lasic, 1992).

The introduction of perfluoroalkylated chains in a surfactant greatly enhances its amphiphilic character, resulting in increased surface activity and hydrophobic interactions. The fluorinated surfactants synthesized in our laboratory combine fluorinated tails with hydrocarbon spacers of diverse length and hydrophilic heads which vary in size, shape, charge, nature and hydrophilic character, and include sugars, polyols, sugar phosphates, amino acids, phosphocholines, phosphatidylcholines, polyethylene glycol phosphates, Tris or lactobionamides (Riess, 1988, 1994a,b; Greiner et al., 1993; Riess et al., 1989; Riess and Krafft, 1994).

Among these surfactants, the perfluoroalkylated phosphatidylcholines (Fig. 1) are promising in view of their low acute toxicity (Santaella et al., 1991a). They form liposomes with much longer room temperature shelf stability than those formulated with their hydrocarbon analogs (Riess et al., 1991; Santaella et al., 1991b). Interestingly, the clearance of these fluorinated liposomes from circulation was found to be significantly retarded as compared to that of conventional liposomes (Santaella et al., 1993). This was attributed to the fluorinated lipophobic core present inside the liposomal membrane which can reduce the adsorption and anchoring of lipophilic plasma proteins onto the liposome's surface, thus hindering their recognition and uptake by the MPS. The same factors are thought to be responsible for the high in vitro drug encapsulation stability observed for such liposomes (Frézard et al., 1994a,b).

Single-chain phosphocholine-based surfactants (Fig. 1), although their acute toxicity is higher, are ideal models for determining the impact of a fluorinated tail on the colloidal behaviour of amphiphiles. They allow the formation of very stable fluorocarbon emulsions (Krafft et al., 1990), liposomes (Krafft et al., 1993) and other supramolecular structures (Giulieri et al., 1994), in spite of having only a single hydrophobic tail.

We report here the effect of various fluorinated amphiphiles on the in vitro phagocytic uptake of calibrated polystyrene microspheres by mouse peritoneal macrophages. The amphiphiles investigated (Fig. 1) differ in the nature of their polar head-group: phosphocholine (PC), *N*-ethylphosphocholine (PCEt), or polyethylene glycol phosphate (PPEG) and in their hydrophobic chains (one or two or no fluorinated chains, diverse length of hydrocarbon spacer) (Krafft et al., 1990; Santaella et al., 1991a; Gaentzler and Vierling, 1993a,b). Their effect on phagocytosis was compared to that resulting from coating the microspheres (i) with related hydrogenated surfactants, i.e., DPPC, Pluronic® F-68, egg yolk phospholipids (EYP), decyl- and pentadecylphosphocholines (Engel et al., 1989), and (ii) with distearoylphosphatidylethanolamine polyethylene glycol (DSPE-PEG 5000) (Blume and Cevc, 1992), a surfactant which is used for the formulation of stealth liposomes. The in vitro phagocytosis model used in this study was shown to correlate satisfactorily with the in vivo phagocytic uptake by the MPS (Illum et al., 1986).

2. Materials and methods

2.1. Materials

The perfluoroalkylated surfactants utilised (Fig. 1) were synthesized in the laboratory. Egg yolk phospholipids (EYP) was obtained from Asahi Glass, Pluronic F-68 from Serva, Dextran 20 and PEG 2000 from Fluka. Fluorescent polystyrene microspheres (2.5% suspension, 0.995 μm in mean diameter, labelled with isothiocyanate fluorescein) were purchased from Polyscience Inc. The products for cell cultures were from Gibco. DSPE-PEG 5000 was a gift from Professor G. Cevc, Munich.

2.2. Coating of the microspheres

A suspension of microspheres (2.5% w/v) and an equal volume of the surfactant solution to be tested (0.5–2.5% w/v) were mixed using a bath sonicator for 30 min and then incubated at room temperature for 24 h. For the phospholipids, the dispersion of surfactant and microspheres was further sonicated (Branson sonifier, Branson Sonic Power Co.). Each preparation was then passed through a Sepharose CL-4B column to remove excess surfactant.

The surface charge on the uncoated and coated microspheres was determined by laser Doppler anemometry (Zetasizer 3, Malvern Instruments, Malvern, UK). The suspension of microspheres was diluted with a 10^{-2} N KCl solution at pH 7 and 20° C. The electrophoretic mobility and the zeta potential reported are the mean of three measurements.

The coating of the microspheres by the phospholipids was assessed using aqueous two-phase partitioning experiments: 0.3 ml of the microsphere suspension was vigorously mixed with 3 ml of an aqueous two-phase system solution (20% w/v dextran 20, 15% w/v PEG 2000, 0.01 M phosphate buffer (PBS) and 0.05 M NaCl). The resulting mixture was allowed to settle by gravity for 3 h. The amount of microspheres present in each phase was determined by absorption measurements at 450 nm using a Pye Unicam PU 8650 Philips spectrophotometer, or by fluorescence using an LS50B Perkin Elmer spectrofluorimeter (excitation 458 nm and emission 540 nm). The partitioning of the perfluoroalkylated phosphatidylcholines between the aqueous two-phase system was also analyzed by ^{19}F-NMR using a Bruker AC-200 spectrometer. With this PEG/dextran system the method cannot be used to assess the coating of microspheres with PEG surfactants owing to specific interactions between the ethylene oxide units of the PEG-rich top phase with those of the PEG surfactants (Müller, 1991).

For the phagocytosis experiments the coating of microspheres was achieved with 1.5% w/v solutions of the surfactants, a concentration which was deemed to be largely sufficient to ensure complete coating of the microspheres (see section 3).

2.3. Serum protein adsorption

A suspension (0.5 ml) of uncoated or coated microspheres containing approx. 2.3×10^6 particles (estimated from the equation: $6C10^{10}/d\pi D^3$, where C is the w/v %, d the density of polymer in g ml^{-1} (1.05 for polystyrene) and D the diameter (in mm) of the latex particles) was incubated with 50 μl of a 50% swine serum solution for 1 h. The preparation was then centrifuged for 15 min at 10000 rpm. The supernatant was removed and the pellet washed with water. The pellet was resuspended in 1 ml of 2×10^{-2} N NaOH, and 20 μl of the resulting solution were analyzed spectrophotometrically at 562 nm for adsorbed proteins using the bicinchroninic acid protein assay reagent. Bovine serum albumin was used as an external standard and all samples were measured against a blank containing 20 μl of 2×10^{-2} N NaOH. Each measurement was made in triplicate and the amount of proteins adsorbed on the uncoated or coated microspheres was expressed in mg of proteins for 2.3×10^6 microspheres.

2.4. Phagocytosis experiments

Male mice (IOPS, OF1) weighing 20–22 g were killed by dislocation of the neck and 5 ml of E199 tissue culture medium, supplemented with 10% swine serum, sodium bicarbonate, an antibiotic (penicillin, streptomycin) and heparin, were injected into the peritoneal cavity along with a small volume of sterile air. The macrophage-containing suspensions from several animals were withdrawn and pooled in a sterile container at 0° C. The macrophages were counted with a haemocytometer and their viability (in the range of 95–97%) was assessed by the trypan blue exclusion test. The macrophage suspension was adjusted to 10^6 cells in 30 mm Petri dishes and their adherence ensured by incubating the plates in 95% air/5% CO_2 at 37° C for 3 h. The non-adherent cells were eliminated by washing the plates once with sterile PBS. The plates were then incubated in 95% air/5% CO_2 at 37° C for 24 h with

1.25 ml of the same medium as above, after which this medium was removed and the cells washed once with sterile PBS. 2.5 ml of the tissue culture medium, with or without 10% swine serum added, containing the uncoated or coated microspheres (their number being adjusted to 20 particles/macrophage) were added to each plate and the plates were incubated at 37° C in 95% air/5% CO_2 for 1 h. The medium was then removed, the plates washed twice with PBS and the cells stained with Wright's colorant.

Each surfactant was tested three times in triplicate and the number of microspheres phagocytized per 100 macrophages was estimated using fluorescence microscopy (500 ×). The results were expressed as the relative percentage of phagocytosis, i.e., the percentage of uptake with respect to that of uncoated microspheres (taken as 100%). The mean of the relative percentage of phagocytosis is given for each surfactant in Table 1 along with the standard error. A statistical analysis of the data (*t*-test, Student Fisher) was made in order to compare among themselves the relative percentages of phagocytosis of the microspheres coated with the various surfactants (Fig. 3). The regression lines for the relation between the relative percentage of phagocytic uptake and the amount of proteins adsorbed (Fig. 4) were computed using Statgraphics.

3. Results

3.1. Surface charge

Except for the perfluoroalkylated phosphatidylcholines, the microspheres, when coated with the surfactants listed in Table 1, showed a significant decrease in electrophoretic mobility as compared to uncoated microspheres. The zeta potential (in absolute values), as calculated from the electrophoretic mobilities, decreased with increasing concentration of the surfactant until a plateau was reached (Fig. 2), indicating that maximal coating was attained for a concentration of approx. 1.5% w/v. The most effective surfactants in terms of zeta potential reduction were DSPE-PEG 5000 and Pluronic F-68. Surprisingly, in the single-chain fluorinated surfactant series and for a given hydrophobic chain (F8C5), the nature of the polar head-group, PC or PEG, seemed to have little effect on the zeta potential; the latter was then similar to that obtained for microspheres coated with EYP. For these single-chain surfactants, an increase in length of the hydrophobic chain resulted in a decrease in zeta potential in both the fluorinated and hydrogenated series.

With the perfluoroalkylated phosphatidylcholines, the coated microspheres displayed elec-

Table 1
Electrophoretic mobilities (EPM) and zeta potential (ZP) for bare microspheres and microspheres coated with different materials in 10^{-2} N KCl, pH 7, 25° C (in μg of adsorbed proteins for 2.3×10^6 uncoated or coated microspheres)

Entry	Coating materials	EPM (μm cm S^{-1} V^{-1})	ZP (mV)	Adsorbed proteins (μg) (± S.D.)
1	None	−3.84	−54.8	85.3 ± (1.2)
2	EYP	−2.91	−41.7	78.5 ± (1.2)
3	Pluronic F-68	−1.21	−17.3	31.9 ± (1.7)
4	DPPC	−3.11	−44.4	77.8 ± (1.1)
5	DSPE-PEG 5000	−1.06	−15.2	41.9 ± (0.9)
6	C10PC	−3.02	−43.2	74.3 ± (2.0)
7	C15PC	−2.50	−35.7	76.9 ± (1.3)
8	F8C2PC	−3.11	−44.5	39.4 ± (1.1)
9	F8C5PC	−2.80	−40	39.7 ± (2.2)
10	F8C11PC	−1.86	−26.6	79.4 ± (2.3)
11	F8C5PCEt	−2.93	−41.9	59.4 ± (2.1)
12	F8C5P[PEG 750]$_2$	−2.99	−42.7	44.4 ± (0.5)
13	F8C5PPEG 2000	−2.52	−36	42.2 ± (1.4)
14	DF6C10PC	−3.65	−52.1	–
15	DF8C4PC	−3.56	−50.9	–

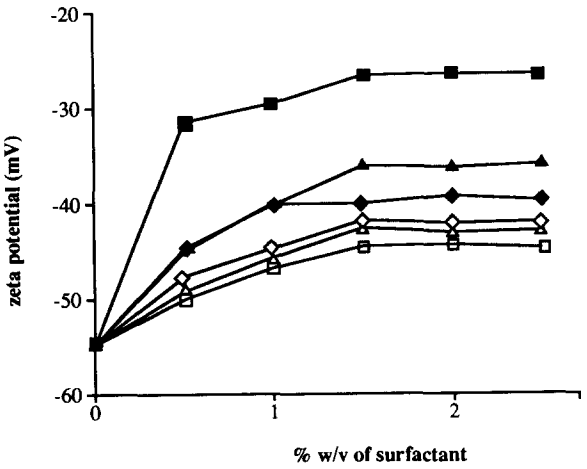


Fig. 2. Evolution of zeta potential with percentage (w/v) of surfactant coating the microspheres: (□) F8C2PC, (◆) F8C5PC, (■) F8C11PC, (◇) F8C5PCEt, (△) F8C5P[PEG 750], (▲) F8C5PPEG 2000.

trophoretic mobilities that were higher than with EYP and nearly the same as for uncoated microspheres, suggesting that coating of the microspheres was only partial. This was confirmed by partitioning experiments using an aqueous two-phase PEG/dextran system. In this system, uncoated microspheres accumulate in the PEG-rich top phase while the coated ones are found in the dextran-rich bottom phase and at the interface. Table 2 shows that a large proportion (49 and 58%, respectively) of the microspheres, when coated with DF6C11PC or DF8C5PC were removed into the top phase. None of the other surfactants with a PC head-group went into the top phase. When the same experiment was performed with a dispersion of these perfluoroalkylated phospholipids, no surfactant was detected by ^{19}F-NMR in the top phase, thus eliminating the possibility of interaction of these surfactants with the PEG-rich top phase. The difference in behaviour of DF6C11PC and DF8C5PC as compared to the other PC surfactants may arise from poorer affinity for the microspheres' surface and/or more pronounced tendency to form liposomes. These two surfactants could therefore not be tested for phagocytosis.

3.2. Serum protein adsorption

The amount of pig serum proteins adsorbed on the coated microspheres (Table 1) increased along the following sequence: Pluronic F-68 < F8C*m*PC (m = 2, 5) ~ DSPE-PEG 5000 ~ F8C5PPEG < F8C5PCEt < C10PC ~ C15PC ~ F8C11PC ~ DPPC ~ EYP. It was the lowest for those microspheres which also displayed the lowest phagocytic uptake in serum (next section). As seen in Fig. 4, a significant correlation was found between the amount of proteins adsorbed and the relative percentage of uptake ($r = 0.67$; $p = 0.0006$ for 95% confidence).

The finding of significantly lower protein adsorption in the case of coatings made from F8C2PC and F8C5PC as compared to hydrogenated compounds of similar length is noteworthy. It demonstrates that the fluorocarbon termi-

Table 2
Partitioning of surfactant-coated microspheres in an aqueous two-phase dextran 20/PEG 2000 system (mean of three experiments)

Coating materials	Top phase (%)	Interface (%)	Bottom phase (%)
None	91.5 ± (4)	8.5 ± (3.5)	–
EYP	–	40.5 ± (6)	59.5 ± (1.5)
DPPC	–	52.5 ± (2.5)	47.5 ± (4)
C10PC	–	18.5 ± (4.5)	81.5 ± (5.5)
C15PC	–	14.5 ± (2)	85.5 ± (4)
F8C2PC	–	11.5 ± (3)	88.5 ± (2.5)
F8C5PC	–	17.5 ± (5)	82.5 ± (3.5)
F8C11PC	–	14 ± (5)	86 ± (3)
DF6C10PC	49 ± (3.5)	33.5 ± (2.5)	17.5 ± (5)
DF8C4PC	57.5 ± (2)	31 ± (5)	11.5 ± (2)

nation has a definite impact on protein adsorption. These two compounds have the longest fluorinated chain and shortest hydrocarbon spacer among the PC derivatives investigated. Increasing the lipophilic character by lengthening the hydrocarbon spacer, as in F8C11PC, or by replacing a methyl by an ethyl group in the polar head, as in F8C5PCEt, resulted in increased protein adsorption and phagocytic uptake (next section). The fact that almost the same amount of protein adsorption and same percentage of phagocytic uptake were found for microspheres coated with F8C11PC and with the hydrogenated PC derivatives indicates that the long hydrocarbon spacer present in F8C11PC is masking the fluorinated portion inside the surfactant film.

3.3. Phagocytosis

The in vitro phagocytic uptake experiments of surfactant-coated microspheres by mouse peritoneal macrophages were run at 37° C in the absence and presence of serum. Coating the microspheres with any of the surfactants listed in Fig. 1 resulted in a decrease in the relative percentage of phagocytosis from at least 20 to almost

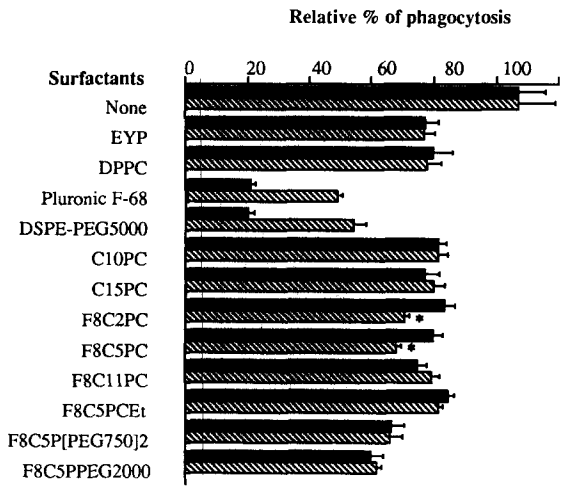


Fig. 3. Relative percentage of phagocytosis of surfactant-coated microspheres: without serum (filled bars), with serum (hatched bars); three experiments per data point; * difference between phagocytosis without and with serum is significant with $p < 0.05$, t-test (Student Fisher).

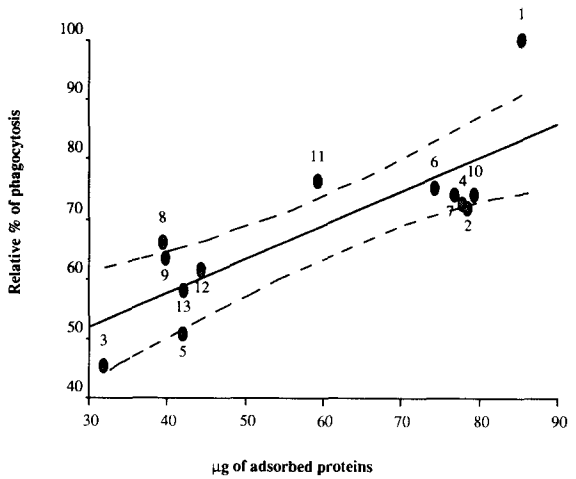


Fig. 4. Relative percentage of phagocytosis as a function of the amount (μg) of proteins adsorbed on the uncoated and coated microspheres. The straight line represents the best fit ($r = 0.67$, $p = 0.0006$). The envelope represents 95% of confidence. Numbers correspond to the entry numbers of Table 1.

80% in the absence of serum, and from approx. 50 to 80% in the presence of serum (Fig. 3). In the absence of serum, this percentage was lowest for Pluronic F-68 and DSPE-PEG 5000 and, to a much lesser extent, for the two fluorinated PEG surfactants.

The presence of serum had in most instances no effect on the relative percentage of uptake except in two cases: (i) for the two fluorinated PC surfactants F8C2PC and F8C5PC, for which serum induced a slight but significant decrease of phagocytic uptake; and (ii) for Pluronic F-68 and DSPE-PEG 5000 for which it induced, in contrast, a more than 2-fold increase in the relative percentage of uptake. Whereas there is no significant difference in uptake within the PC-surfactant series in the absence of serum, in serum the phagocytic uptake of the microspheres coated with F8C2PC or F8C5PC became lower than when coating was achieved with F8C11PC or with any of the hydrogenated PCs. The uptake of microspheres coated with F8C2PC or F8C5PC in serum was, surprisingly, comparable to that obtained with the two fluorinated PEG surfactants, indicating that introducing a fluorinated chain, provided the hydrocarbon spacer is short, may induce a similar effect on phagocytosis to that of

introducing hydrophilicity and steric bulkiness as with PEG chains. Coating the microspheres with either the neutral F8C5P[PEG 750]2 or the anionic F8C5PPEG 2000 surfactants resulted in very similar phagocytic uptakes. Replacing a methyl group by an ethyl group in F8C5PC, to yield F8C5PCEt, had almost no effect on phagocytosis in the absence of serum. In serum, however, the uptake was lower with the former compound, indicating that an increase in lipophilic character may induce an increase in phagocytosis.

4. Discussion

The control of the phagocytosis of colloidal carrier systems is a major challenge in biomedical research. Many parameters, including size, charge, rigidity, hydrophilicity, surface availability and molecular packing, can influence the recognition and phagocytosis of foreign particles by the MPS (Illum et al., 1986; Kawaguchi and Koiwai, 1986; Allen et al., 1991; Blume and Cevc, 1992). The contribution of each one of these often interrelated parameters is, however, far from being clearly established yet.

Where the fluorinated PEG surfactants are concerned, our results are in line with diverse reports which show that bulky, hydrophilic materials such as polyoxyethylene can reduce the opsonization of particles and delay their phagocytosis by peritoneal macrophages in vitro (Illum and Davis, 1984). In the absence of serum the lowest phagocytosis of coated microspheres was indeed obtained with the fluorinated surfactants F8C5PPEG 2000 and F8C5P[PEG 750]2. However, phagocytic uptake remained much higher than with Pluronic F-68 or DSPE-PEG 5000-coated microspheres. In the presence of serum, not only both fluorinated PEG surfactants, but also the non-pegylated fluorinated surfactants F8C2PC and F8C5PC became almost as effective as DSPE-PEG 5000 in reducing phagocytosis. It is noteworthy that the neutral F8C5P[PEG750]2 and anionic F8C5PPEG 2000 behaved very similarly, indicating that the negative charge in the latter is hidden by the long and mobile PEG chain.

Although coating with Pluronic F-68 or DSPE-PEG 5000 led to the lowest zeta potentials and phagocytosis, a correlation between surface charge and phagocytosis could not be established. In the absence of serum the microspheres coated with the two fluorinated PEG surfactants and with the non-pegylated PC derivatives displayed similar zeta potentials but the former were less phagocytized. One also observes in the PC series that a large decrease in surface charge, as with F8C11PC, is not necessarily accompanied by a decrease in phagocytic uptake. These observations are in agreement with data indicating that surface charge is not necessarily the determining factor in particle phagocytosis (Van Oss, 1978; Privitera et al., 1994).

On the other hand, a significant correlation between phagocytosis and opsonization (Fig. 4) was found, indicating that protein adsorption is an important factor in the phagocytosis process. Although the relative percentage of phagocytosis is in most cases not affected, or increases when serum is present, it appears nevertheless that the lower the amount of proteins adsorbed on the microspheres the lower is the phagocytic uptake.

The most interesting and unexpected finding of this study is certainly that the relative percentage of phagocytosis decreases in the case where the coating agent is F8C2PC or F8C5PC when going from buffer to serum. It then became comparable with coating with DSPE-PEG 5000. This peculiar behaviour indicates that factors other than particle size, surface charge or opsonization (in terms of amount of adsorbed proteins) play a role in macrophage recognition and phagocytic uptake. A better understanding of this phenomenon may require identification of the nature of the adsorbed proteins on the surface of the microspheres for each specific coating.

The decrease in macrophage uptake of microspheres coated with the fluorinated phosphocholine-derived single chain amphiphile F8C2PC and F8C5PC illustrates the impact fluorinated chains can have on particle recognition and phagocytosis. This arises most likely from the lipophobic barrier formed by the perfluoroalkyl chains within the surfactant film (Riess et al., 1991; Riess, 1994a,b). This barrier is thought to hinder to some degree the adsorption and an-

chorage of proteins and subsequent uptake by macrophages. The effect decreases and indeed disappears when the fluorinated segment is moved further away from the surface (as with F8C11PC) or is absent (as with C10PC or C15PC), and/or if the lipophilic character of the amphiphile's polar head is increased (as with F8C5PCEt). These results are in line with a previous study which showed that, in the presence of serum, phagocytosis of microspheres coated with fluorinated telomeric (acrylate) surfactants derived from tris(hydroxymethyl)aminomethane was lower than with their more lipophilic analogs (Privitera et al., 1994). They are also in line with the slower MPS uptake found for fluorinated liposomes made from the double-chain phosphatidylcholines DF8C5PC and DF6C11PC, as compared to conventional ones (Santaella et al., 1993). Unfortunately, the inability of the latter compounds to completely cover the microspheres prohibited the examination of their effect on phagocytic uptake. Where the three pegylated surfactants investigated are concerned, the large differences in their structure did not allow the assessment of whether or not there was an effect of the fluorinated chain on phagocytosis.

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