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Dynamics of antibody- and lectin-mediated endocytosis of hapten-containing liposomes by murine macrophages

The uptake by murine macrophages of liposomes, exhibiting one of a variety of haptenic groups on their surfaces, was greatly enhanced by the addition of an intact antibody or a lectin specific for the incorporated hapten. The uptake of untreated liposomes was slow and linear over long periods, whereas upon addition of the antibody or lectin, over 30-fold increase in the maximal rate of uptake was observed. The process reached a plateau after 90–120 min. The interaction of the antibody- or lectin-treated liposome with the macrophages apparently resulted in an active endocytosis of the vesicles. As observed by fluorescence microscopy, the distribution of a water-soluble fluorescent, intraliposomal marker had a granular intracellular pattern in treated cells. The uptake was sensitive to azide and the liposome constituents could not be detected at the cell surface. The size of the liposomes as well as the state of stimulation of the macrophages (thioglycollate stimulated *vs.* normal) did not seem to have a major effect on the phagocytic process. The time required to reach the plateau in uptake was independent of liposome composition or antibody concentration and is, apparently, an intrinsic property of the cells. The implication of this phenomenon on the dynamics of the relevant macrophage receptors is discussed.

1 Introduction

The wide interest in the interaction of cells with lipid vesicles was motivated in recent years mainly by studies which demonstrated the feasibility of using liposomes as carriers of drugs, enzymes, *etc.* into cells [1–4]. It has been shown that under specific conditions, liposome-cell interaction may take place both *in vitro* [5] and *in vivo* [6], with the subsequent introduction of their contents into the cytoplasm. In view of this information it seemed of interest to investigate the mode and mechanism of the reaction of liposomes with various cells, and the factors that may modulate this interaction.

The contact between a cell and a liposome may lead to one or several of the following consequences. (a) The liposome may be physically *adsorbed* on the cell membrane, and may be retained on the cell surface either intact [7] or, in turn, undergo lysis [8–10]. (b) The liposome may be *endocytosed* [11], and thus the entire liposome is engulfed by the cell and

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Abbreviations: DNP: 2,4-Dinitrophenyl **MΦ:** Macrophages **HBSS:** Hanks' balanced salt solution **FCS:** Fetal calf serum **ANTS:** Aminonaphthalene-3,6,8-trisulfonate **DPX:** Bis-pyridinium-p-xylene **SBA:** Soybean agglutinin **PBS:** Phosphate-buffered saline **SDS:** Sodium dodecyl sulfate

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enclosed in a phagocytic vacuole inside it. (c) The liposome may *fuse* with the cell membrane, a process which may result in the incorporation of its lipid moiety into the cell membrane and its aqueous interior into the cytoplasm [12, 13]. (d) The liposome may *exchange lipids* with the cell membrane, directly or *via* an intermediate carrier [14].

The factors that determine which of the above processes will take place include both the type of cells or organism involved, and the physicochemical characteristics of the liposomes. Thus, it has been shown that liposomes with different lipid composition may vary in their behavior after *in vivo* injection. This can be manifested in their rate of clearance from the circulation as well as in their tissue distribution [15]. The electrical charge and fluidity of the liposome membrane may also be of influence [16, 17].

Systematic manipulation of the liposome membranes, either by changing the lipid composition, or by addition of extraliposomal factors, may result in a pronounced effect on the fate of the liposomes, both *in vivo* and *in vitro*. Thus, it has been reported that desialylated fetuin incorporated into the liposomal surface mediates an enhanced uptake of liposome-associated drugs by liver cells *in vivo* [18]. Weissman and coworkers were also able to increase the uptake of liposomes into phagocytes by a factor of 2 by pretreatment with aggregated IgM [19, 20]. In this latter experiment it is conceivable that the aggregated immunoglobulin was bound to the liposome *via* a portion of its Fc region [20], whereas other Fc regions of the same aggregate were available for the subsequent binding to Fc receptors on the cell, triggering the endocytosis.

The process of opsonization, namely the uptake of particulate antigens in general, by several types of cells, including macrophages (MΦ), has been described a long time ago [21, 22]. It has been shown to be mediated primarily by the Fc region of specific antibodies of the IgG class [23], as one of the manifestations of antigen-antibody interactions. In this respect, liposomes may exert a pattern of behavior similar to that of a particulate antigen with a desired antigenic specificity, dictated by their lipid contents as has been recently described [24].

In the present study, we show that the rate of phagocytosis of liposomes displaying a distinct antigenic grouping on their membrane, by murine MΦ, is strongly enhanced by the addition of relevant antibodies or a specific lectin to the liposomes prior to the phagocytic process.

2 Materials and methods

2.1 Cells

Peritoneal MΦ were harvested from nonstimulated or from thioglycollate-stimulated (BALB/c × C57BL)F_1 mice [25–26]. The cells were collected in Hanks' balanced salt solution (HBSS), free of phenol red, at 4 °C, and washed. Unstimulated mice yielded about 5×10^6 peritoneal cells/mouse with about 30–35% MΦ (determined by both morphology and phagocytic capability). Thioglycollate-stimulated mice yielded, each, about 30×10^6 peritoneal cells, with 80–85% MΦ. The cell suspension was adjusted to a concentration of 5×10^5 MΦ/ml, in HBSS containing 5% fetal calf serum (FCS, Microbiological Associates, Jerusalem, Israel), and 2-ml aliquots were applied to 10 × 35 mm culture plates (Falcon Plastics, Oxnard, CA). The cultures were incubated at 37 °C in a humid atmosphere of 5% CO_2 and 95% air for 16–20 h. Then, the monolayers were extensively rinsed with phosphate-buffered saline (PBS), to remove non- or loosely adherent cells, and supplemented with fresh medium.

2.2 Liposomes

The liposome systems used throughout these experiments were prepared essentially as described previously [27, 28]. A solution in chloroform : methanol (1 : 1) of phosphatidylcholine, cholesterol, dicetylphosphate and α-tocopherol in the molar ratios 4.0/3.0/0.1/0.5 (basic lipid mixture) was prepared and stored at − 20 °C. This solution was combined with different amounts of the various lipid haptens (see below) in $CHCl_3$: MeOH (1 : 1).

The appropriate lipid mixtures in the organic solvent (2.0 mg of total lipid) in a test tube were evaporated under nitrogen and dried under vacuum for several hours. To this was added 0.2 ml of a solution 54 mM with respect to aminonaphthalene trisulfonate (ANTS) and bis-pyridinium-p-xylene (DPX) and of equivalent tonicity to the buffer A (0.1 M Tris-HCl, pH 7.5, containing 0.025 M NaCl and 0.025 M KCl) [28]. This solution was prepared by dissolving 163 mg of the ANTS-DPX complex in 3 ml of water, adjusting the pH to 7.4 and the volume to 3.5 ml followed by 1.5 ml of buffer A. The lipid suspension was mixed with a micromagnetic bar overnight in an N_2 atmosphere in the dark. It was then vortexed (the magnetic bar present) for 5 min and loaded on a Sepharose 6B column to remove the external untrapped DPX and ANTS. Since Sepharose 6B binds lipids, the above column was prepared as follows: the Sepharose 6B was first washed with water, then with buffer A, then with a suspension of phosphatidylcholine liposomes, followed by buffer A. The liposomes eluted in the void volume (0.5 ml) were collected and stored under N_2 in the dark at room temperature until used.

In the case of sonicated liposomes, sonication was performed for 45 min per 0.2 ml in an ice bath sonicator (Laboratory Supplies Company, Hicksville, NY) at 80 W. In a few preparations, the sonicated liposomes were passed through a Sepharose 2B column yielding two peaks that exhibited a similar behavior in uptake experiments.

In addition to the standard lipid mixture (Mix A), the liposomes contained 10% (w/w) of one of the following haptenic groups: dinitrophenylated phosphatidyl ethanolamine (DNP-PE), prepared according to Kinsky's method [29], ganglioside GM_1 (purified from bovine brain according to Svennerholm [30]) and ganglioside GM_2 (purified from Tay-Sachs brain according to Svennerholm [30]). Both gangliosides were purified by column chromatography and subsequent preparative thin-layer chromatography. Sulfatide was purified by Dr. Y. London, and total cerebrosides were purchased from Sigma Chemical Co., St. Louis, MO. Each one of the gangliosides migrated as a single band in thin-layer chromatography, indicating purity. In the inner aqueous phase of the liposomes was entrapped a complex of the water-soluble fluorophore, ANTS and the quencher DPX [28]. Measurements of ANTS fluorescence were performed in a Perkin-Elmer spectrofluorometer MPF-44A with $\lambda_{exc.}$ 358; $\lambda_{emm.}$ 520 nm.

2.3 Antibodies and lectin

Anti-DNP antibodies were raised in rabbits by injection of DNP-bovine serum albumin (DNP-BSA). Antibodies were purified on a Sepharose-bound DNP-lysine. The antibodies obtained had an average affinity of 5×10^8 M^{-1} as determined by the method of fluorescence quenching using ε-DNP-lysine [31]. Antisera to sulfatide and to pure gangliosides GM_1 and GM_2 were raised in rabbits by injections of the relevant glycolipid (5 mg) complexed with an equal amount of methylated bovine serum albumin. Cerebroside was similarly injected after complexation with BSA. Antisera were collected after a series of three injections at 21-day intervals, and their activity was estimated by the complement-mediated lysis of liposomes, as described previously [27]. Soybean agglutinin (SBA), free of aggregates, was a gift from Prof. N. Sharon, the Department of Biophysics in the Weizmann Institute.

2.4 Liposome uptake assay

Liposomes containing the various lipophilic haptens were incubated with the relevant antibodies, antiserum, lectin, or with normal serum for 15 min at room temperature. Unless specified, the concentration of antibody used was selected to be subagglutinating (agglutination detected by microscopy). Aliquots of these liposomes (usually 30 μl containing 130 μg total lipid) were added to 10^6 cultured MΦ in 2 ml HBSS + 5% FCS. After various incubation periods, individual monolayer plates were rinsed extensively and lysed by addition of 1 ml of 0.1% sodium dodecyl sulfate (SDS). The uptake of liposomes was assayed by the extent of fluorescence in the lysates.

The results are expressed in percent uptake, namely, percent of the total entrapped fluorescence, calculated as following:

$$\% \text{ Uptake} = \frac{\text{Fluorescence of lysate of cells} \times 100}{\text{Fluorescence of SDS-treated liposomes} - \text{fluorescence of intact liposomes}}$$

All fluorescence measurements were carried out in a volume of 1 ml. The fluorescence of the intact liposome preparation was subtracted since the uptake of the free fluorophore by the cells is negligible (less than 1%).

3 Results

3.1 Antibody-mediated uptake of liposomes

All the liposome preparations underwent slow uptake by the cultured MΦ. However, pretreatment of the liposomes with the relevant antibodies resulted in a marked augmentation of their uptake by the cells. This phenomenon is demonstrated for five different antigenic systems which show essentially the same behavior (Fig. 1). This process is strictly specific: it requires the presence of the relevant antigen in the liposome membrane, as demonstrated by the finding that none of the antibodies or antisera enhanced the uptake of "non-antigenic" liposomes (containing only Mix A in their membrane) (Fig. 1, bottom right), or of liposomes containing irrelevant antigen. Similarly, nonimmune serum or its IgG fraction did not bring about an enhancement of the uptake of any of the liposomes.

The augmentation of endocytosis requires the presence of the intact antibody molecule. Thus, in the case of the DNP system, where purified antibodies were used in the experiment, the $F(ab')_2$ fragments prepared from them did not mediate any increase in the uptake (Fig. 1, top left), although they retained the full antibody activity, as demonstrated by various methods, including their capability to agglutinate the liposomes. The phenomenon is thus dependent on the presence of the Fc region in the antibody.

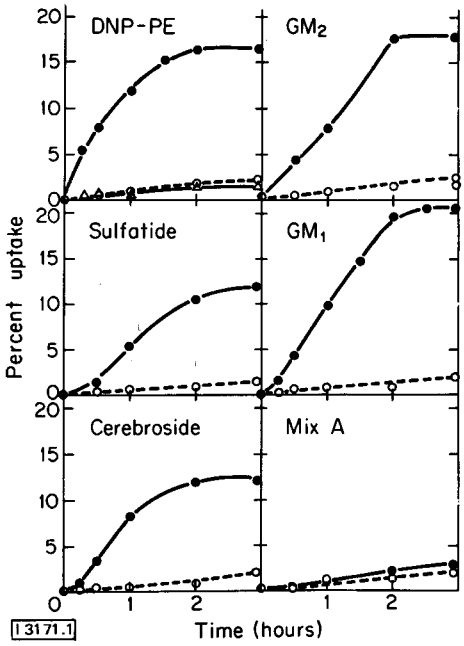

Figure 1. Enhancement of endocytosis of lysosomes by specific antibodies. Liposomes containing the specified lipid in their lipid bilayer were preincubated for 15 min with the respective antiserum (●—●) or normal rabbit serum (○---○), prior to the incubation with the MΦ; 30 μl of liposomes, containing 130 μg of lipids were used in each experiment. In the case of DNP-PE, purified antibodies (○---○) or their $F(ab')_2$ fragment (△---△) were used in the interaction with the liposomes.

The kinetics of uptake of the antibody-treated liposomes showed in some cases an initial short lag period, followed by rapid uptake, with a maximal rate of 0.3–0.5%/min, for up to 2 h. After that time, a plateau in the uptake was reached at a level of 12–20% uptake (17–26 μg lipid). Untreated liposomes or liposomes incubated with nonimmune sera were taken by the cells at a slow linear rate of about 0.01%/min, or less.

The concentration of antibodies used in the pretreatment of the liposomes had a profound effect on the rate of uptake and the uptake level at the plateau (Fig. 2), but a decrease in the concentration of antibodies by a factor of 2.5 did not change the length of time required to reach the plateau, namely 90–120 min. Further increase of the antibody concentration used for pretreatment of the liposomes was not employed in this experiment, since it resulted in agglutination of the liposomes as detected by light microscopy. Since the size of the liposomes is about 1 μ, this seems to be a reliable method, and any aggregates formed could be easily visible. No such aggregates were detected in the range of antibody concentration used.

FCS present in the incubation media, although increasing the density of the solution and consequently the buoyancy of the

Table 1. Effect of FCS on liposome uptake

Serum concentration (%)	% Uptake after 30 min	90 min
0	9.4	16
2.5	8.0	24
5.0	7.5	21
7.5	5.2	20
10	4.0	21

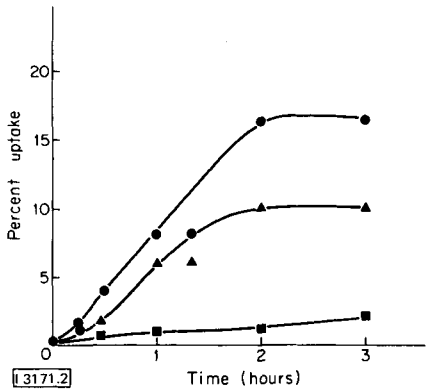

Figure 2. Effect of antibody concentration on the enhancement of phagocytosis of GM_1 liposomes by the MΦ. Preincubation of 30 µl liposomes was carried out with 50 (●), 20 (▲) or 10 µl (■) of antiserum.

liposomes, did not affect the extent of uptake. This was demonstrated in an experiment carried out in the presence of various concentrations of FCS within the range of 0–10%. The results (Table 1) show a slight decrease in the initial uptake with an increasing concentration of FCS, but no effect on the uptake after 90 min.

The extent of uptake was shown to be directly proportional to the number of cells in each plate, up to a cell concentration of 2×10^6 that formed a confluent monolayer. Thus, the density of the cells in the culture *per se* does not seem to affect significantly the uptake per cell. We have also studied the effect of the size of the liposomes and of the state of activation of the MΦ on the uptake process. It was observed that addition of sonicated, small liposomes, pretreated with antibodies, to 10^6 cultured MΦ, resulted in a somewhat higher rate of uptake than that of the equivalent amounts of multilamellar liposomes. The difference between the two preparations did not seem to be highly significant, and the uptake value at the plateau was the same for both liposome preparations, about 15% (20 µg lipid). Thioglycollate-stimulated MΦ did not differ significantly from the unstimulated cells in either the rate of uptake or the other characteristics of the process.

3.2 The nature of liposome uptake by macrophages

The association of the liposomes with the MΦ could, in principle, result from at least three types of interactions: (a) an extracellular adsorption on the cell membrane; (b) fusion of the liposome and the MΦ membrane with the subsequent transfer (partial or complete) of their fluorescent contents to the MΦ cytoplasm and (c) an active phagocytosis of the intact liposomes by the MΦ. To distinguish between these possibilities, we have carried out the following experiments.

MΦ on a cover slip were incubated for 30 min with 10% cerebroside-liposomes, preincubated with anti-cerebroside antiserum. They were then rapidly rinsed with fresh medium and observed under the fluorescence microscope. The observed fluorescence was photographed immediately since a rapid bleaching occurred under UV light. The fluorescence was considerably brighter if DPX was not included inside the liposomes. The results (Fig. 3) indicated that the cell-associated fluorescence is concentrated in granular structures, mainly in the perinuclear region of the cell. Careful focusing in the microscope suggests that this fluorescence is almost entirely intracellular.

Using another system, cells incubated with antibody-treated DNP-PE liposomes, did not stain by reacting with rabbit anti-DNP antiserum and rhodamine-labeled goat anti-rabbit IgG. In contrast, trinitrophenylated MΦ treated for 10 min at 25 °C with down to 10 µM trinitrobenzene sulfonate in PBS, pH 7.4, gave a bright immunofluorescent staining under these conditions. Moreover, following the uptake of DNP-liposomes, the cells were not sensitive to anti-DNP and guinea pig complement (as determined by exclusion of trypan blue), nor was it possible to release the cellular associated fluorescence from the MΦ by the treatment. These experiments indicate that the DNP-liposomes are not on the outer surface of the MΦ.

An additional corroboration to the notion that the liposomes were taken up by the cells *via* an active process was obtained

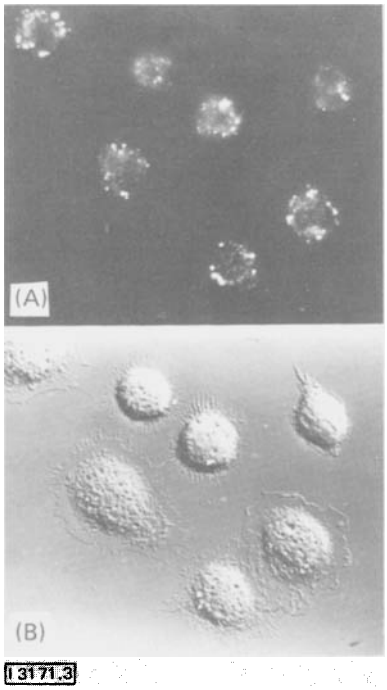

Figure 3. Light micrographs of the MΦ after endocytosis of antibody-pretreated cerebroside liposomes. (A) Fluorescence photomicrograph was taken very shortly after illumination to avoid bleaching; (B) phase contrast micrograph of the same area.

by the capability of azide (5×10^{-3} M) to block completely the uptake process. The same effect was obtained with cytochalasin b in concentration of 10^{-6} M as well. This indicates that not only the antibody-mediated uptake, but also the uptake of untreated liposomes is probably due to an active, energy-dependent process, rather than a passive adsorption. The effect of azide was reversible, and the same culture, following removal of the azide, showed normal uptake properties after 24 h of recovery.

When the measurement of cellular associated fluorescence was performed on intact MΦ without previous disruption of the cells by SDS, most of the fluorophore in the cell was in a quenched state. This may be interpreted as indicating either that the liposomes taken by the cell remain intact at least up to the time of the measurement (2 h), or alternatively, that the liposomes might have been destroyed, but within lysosomes which are too small to allow the dilution required for the abolishment of the quench.

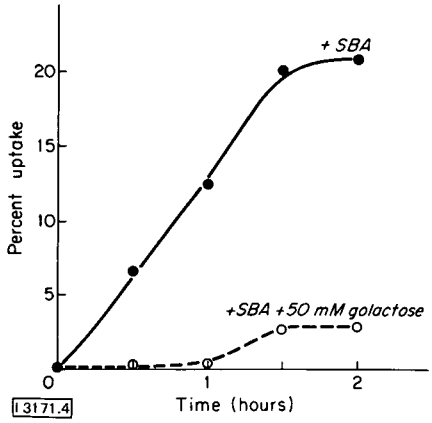

Figure 4. Effect of SBA on the uptake of GM_1 liposomes by MΦ. Uptake in the presence of SBA (●—●), inhibition of SBA-mediated uptake by 50 mM galactose (○---○).

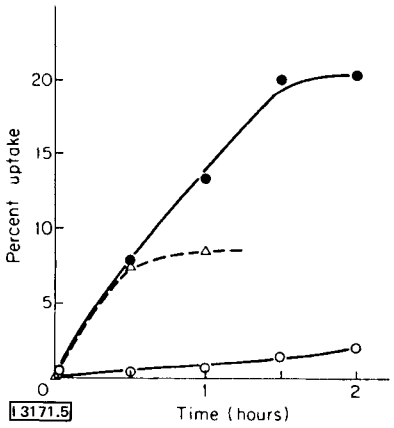

Figure 5. Effect of preincubation with antibody-coated liposomes on subsequent antibody-mediated phagocytosis. Uptake of antibody-coated liposomes by untreated MΦ(●—●); uptake of similar liposomes by MΦ preincubated with antibody-coated nonfluorescent liposomes (△---△); uptake of liposomes pretreated with normal rabbit serum (○—○).

3.3 Lectin-mediated uptake of liposomes

We have tested whether the potentiation of phagocytosis is strictly an immunological phenomenon mediated only by Fc receptors, or whether it is a more general phenomenon that can be effected by other macromolecules. Liposomes containing 10% gangliosode GM_1 were preincubated with an equal volume of a solution of SBA (1 mg/ml; aggregate-free), prior to the addition to the MΦ (SBA has specificity of binding toward galactose, the terminal sugar of GM_1). Fig. 4 shows that the treatment with SBA brings about a marked increase in the uptake of the GM_1 liposomes (compare to Fig. 1, non-treated GM_1 liposomes). The process appears to be azide-sensitive (not shown) and it is specific, as indicated by the strong inhibition caused by galactose. This sugar did not have any inhibitory effect in a nonrelevant uptake system, such as DNP-liposomes: anti-DNP. It should be mentioned that SBA tends to aggregate upon storage [32] and that the aggregated SBA is less effective in potentiating the endocytosis.

3.4 The dynamics of liposome uptake

As already mentioned, the kinetics of the liposome endocytosis process shows, after an initial short lag period, a linear uptake which reaches a maximum after 90–120 min. This plateau in level of uptake could be due to a steady-state condition in which equilibrium is reached between further uptake and release of the fluorophore, or alternatively, it could stem from inhibition of the endocytotic process.

The involvement of fluorescence release was ruled out by the following experiment: MΦ were incubated with antibody-treated GM_1 liposomes for 2 h, and after removal of excess liposomes the cells were supplemented with fresh medium. At different intervals thereafter (up to 3 h) the fluorescence in the culture medium was determined. No significant release of fluorescence was observed, most of it remained intracellular.

Toxic effect of the antibody-treated liposomes on the cells seems unlikely to be the reason for the arrest in liposome uptake at the plateau, since the MΦ remain viable after the endocytic process, retaining their original low capacity to phagocytize untreated liposomes.

The liposomes remaining in the supernatant following 2 h uptake by the MΦ may still be endocytosed by untreated cells, and do not differ significantly from a fresh preparation of antibody-treated liposomes. This rules out the possibility that the liposomes lose their antibodies, or that blocking of the Fc during the incubation period accounts for the observed arrest in the uptake.

It is conceivable, on the other hand, that receptor depletion is the major factor causing the cessation of liposome uptake at a certain maximal value, as suggested by the following experiment: MΦ were incubated for 2 h with 30 µl of antibody-treated liposomes which *did not* contain ANTS-DPX. The monolayers were then rinsed and immediately supplemented with fresh medium containing fluorescent liposomes in 80% of the regular concentration (the concentration which is expected to remain in solution after 2 h uptake. At different intervals thereafter, the cell-associated fluorescence was measured. Control cultures which were not pretreated with nonfluorescent liposomes were incubated in parallel with antibody-

treated or untreated liposomes at the same concentration. The results shown in Fig. 5 demonstrate that the pretreatment with nonfluorescent liposomes resulted in a marked decrease in the extent of uptake. The maximal uptake reached was ca. 8%, but the time required to reach the plateau was much shorter: 30 min instead of 90–120 min in untreated cells. It thus seems that the initial incubation with the liposomes indeed caused an apparent depletion of the relevant receptors.

4 Discussion

The study of cell-liposome interactions in a variety of systems has two major objectives: (a) liposomes appear to be potentially suitable candidates for the delivery of drugs, proteins, *etc.* into living cells [1–4]. Since the structure of liposomes, their size and composition may be manipulated, it enables, in principle, a selection of the optimal liposomes for the delivery of the specific materials into target organs or cells. (b) Liposomes may serve as a useful probe for processes that occur during cell-to-cell interactions or other cell-membrane phenomena.

We have shown that when liposomes containing a variety of haptenic groups are coated with specific antibodies or lectin (SBA), they are rendered very susceptible to phagocytosis by MΦ. This phenomenon requires the intact specific antibody, which suggests that the immunoglobulin molecules serve as bridges that anchor the liposomes to the MΦ membrane *via* the antigen-binding sites and Fc region, respectively.

The phenomenon of Fc-mediated uptake of particulate antigen by MΦ is a well-recognized phenomenon]20]. This approach was utilized by Weissmann et al. [19] for the uptake of liposomes by phagocytes. However, unlike the system presented here, in which the antibodies served for "coating" the liposome by virtue of their specificity, Weissmann et al. reacted aggregated IgM (non-antigen-specific) with the liposomes. The aggregate probably adhered to the liposome membrane in such a manner that sufficient Fc remained free to bind the complex to the phagocyte membrane. The approach presented in this study seems to have two advantages: (a) the relatively high extent of enhancement of phagocytosis and (b) the fact that the rate of endocytosis may be easily manipulated by varying the antibody or antigen concentrations.

The enhancement of liposome uptake by antibodies was achieved with several different haptens in the liposome membrane, including some naturally occurring lipids. It is anticipated that by careful choice of the lipid composition of the liposome as well as the source of the coating antibodies, this mechanism of uptake may be operable also *in vivo* without the risk of harmful immunological reactions such as immediate or delayed-type hypersensitivity. However, it should be realized that this Fc-mediated uptake is restricted only to cells that bear Fc receptors on their surface and show an active endocytotic acitivty. It is thus of importance that other macromolecules which can facilitate the binding of liposomes to cells, such as lectins with the appropriate carbohydrate specificity, exert an effect similar to that of antibodies, as exemplified here by the marked specific enhancement of the uptake of GM_1-containing liposomes in the presence of SBA. The process is thus not strictly immunological but may rather result from "affinity-mediated" association with a variety of cell membrane components.

It should be pointed out that though the present study was performed with "professional phagocytes", the endocytosis of ligands after interaction with the relevant membrane receptors is a general phenomenon observed also with other cells [2]. This process is believed to require the initial redistribution, namely clustering, of the relevant receptors prior to the energy-dependent internalization.

The present study indicates that liposomes may interact with macrophages through at least two different, apparently independent, types of receptors: those that bind the non-opsonized vesicles, and those that bind the Fc. Though we have no direct information on these different phagocytic receptors on a molecular level, or on their specificity, some information on their dynamics may be derived. The non-opsonized untreated, negatively charged liposomes were taken up by the cells at a constant rate for at least 3 h. Longer incubation resulted in some overall decrease in the rate of uptake, which was generally reflected in parallel limited cell death during the assay.

The Fc-mediated uptake, on the other hand, showed different kinetics. This was manifested by a short lag period, a rapid uptake phase and a plateau, normally reached after 90–120 min. It seems that the extent of phagocytosis, expressed as percent of liposomes taken by the cells at the plateau, as well as the lag period, depended mainly upon the amount of liposomes added, and the concentration of opsonizing antibodies. It is conceivable that aggregation of Fc receptors precedes the endocytotic event, and thus the availability of more Fc regions at the liposome surface will affect the rate of the redistribution of the receptors and the subsequent uptake. The time required to reach the plateau, however, seemed to be an intrinsic property of the cells. Our results suggest that following the phase of rapid uptake, there is a decrease in the number of available Fc receptors of the cell membrane, and this probably causes the arrest in endocytosis. This was concluded from the observation that preincubation of the cells with nonfluorescent liposomes drastically reduced the subsequent uptake of fluorescent liposomes (Fig. 5). Whether the apparent decrease in available Fc receptors of the cell surface is a direct result of the interaction with the liposome-associated Fc or, alternatively, stems from a preferential partitioning of Fc receptors (bound and free) into the forming phagocytic vacuole, is not clear. It has been proposed that different membrane components may selectively be included or excluded from the phagosome [33–34]. This is in line with our observation that liposomes sensitized by smaller amounts of antibodies may be taken more slowly, but will nevertheless reach the plateau after the same period of 90–120 min.

Another possibility which has been considered for explaining the phenomenon of the plateau was blocking of the Fc on the antibody-coated liposomes by receptors that had been shed off the cells. A similar mechanism has been suggested to be responsible for the blocking of anti-phagocytic activity of anti-MΦ serum [25], or for the inhibition of T rosetting by supernatants of various cultured human lymphoid lines [35]. However, if this mechanism operates in the system presented here, it has only a limited effect, since the liposomes remaining in solution after the plateau was reached were only slightly less available for uptake by fresh MΦ as compared to untreated vesicles. In addition, it has been observed that MΦ preexposed to liposomes under the above conditions, bind considerably less, or not at all, opsonized sheep erythrocytes at 4 °C.

This supports the notion that indeed a depletion of Fc receptors did occur.

The antibody-mediated interaction of liposomes with cells bearing Fc receptors may also result in consequences other than phagocytosis. It had been reported [8, 36] that antibody-coated liposomes may be bound to, and subsequently lysed by, lymphocytes bearing Fc receptors. However, it seems that for the directed delivery of liposome-entrapped material into cells other than MΦ, different modified schemes may be required. One such possibility may be the use of lectins as demonstrated here. It thus seems that additional studies on the organ-specific and cell-specific targeting of liposomes may open the way towards the use of liposomes as drug carriers for practical therapy.

We wish to express our gratitude to Dr. Carl R. Alving from the Department of Immunology and Hematology, Walter Reed Army Institute of Research, for helpful discussions and for sharing with us his broad experience in related systems.

Received February 3, 1981; in revised form April 3, 1981.

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