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Review article

The potential use of liposome-mediated antiviral therapy

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Summary

The natural targeting of liposomes to cells of the reticuloendothelial system should be exploited to examine whether selective delivery of antiviral or immunomodulatory agents could be beneficial for the treatment of viral diseases. In this review we discuss the potential use of liposomes in the treatment of virus diseases, the targeting of liposome-encapsulated immunomodulators to macrophages in order to render these cells cytolytic for virus-infected cells, and the targeting of liposome-encapsulated antiviral drugs to macrophages to achieve direct suppression of virus replication within these cells.

liposomes; macrophage activation; drug targeting

Introduction

Although viral diseases of man and animals are a major cause of morbidity, mortality, and hence economic loss [1], early hopes for an antiviral panacea have not been fulfilled. The lack of success in the treatment of viral diseases has been due in part to the fact that viral replication is intimately associated with the host cell biosynthetic machinery [2]. Recent discoveries in the molecular biology of virus replication have

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identified several virus-coded functions that are potential targets for selective inhibition by antiviral agents [3]. Moreover, advances in rapid viral diagnosis now allow the identification of a causative agent early in the course of the disease, when most viral replication occurs [4]. The potential for treatment of viral diseases has never been greater.

One of the challenges in the development of effective viral therapeutics is to deliver antiviral agents to the sites of virus replication. This challenge is not unique to viral diseases, and indeed drug targeting has become the focus of extensive study for the treatment of cancer [5,6], fungal [7–9], bacterial [10,11] and parasitic diseases [12]. Recently, substantial effort has been directed at evaluating synthetic phospholipid vesicles, i.e. liposomes, as drug delivery systems that could target therapeutic agents to organ or cellular sites of diseases [13]. The capacity of liposomes to encapsulate a wide variety of hydrophilic or lipophilic biologically active compounds makes them extremely attractive as potential vehicles for drug delivery *in vivo* [14]. Liposomes, however, like other particulate matter, are cleared from the circulation by phagocytic cells of the reticuloendothelial system (RES) [6,15]. Limited transcapillary transport of liposomes following intravenous injection occurs in the liver through open sinusoidal capillaries, but does not occur in the continuous capillaries of the lung. However, liposomes in the lung capillaries are engulfed by circulating blood monocytes which can subsequently migrate to the alveoli to become alveolar macrophages. Although this biological reality presents a major drawback to the use of liposomes for delivering drugs to cells other than phagocytic cells [13,16], it does permit ‘natural targeting’ of drugs to cells of the RES. Studies by Fidler, Poste, and their colleagues [17,18] have taken advantage of this selective delivery of liposomes to cells of the monocyte–macrophage series and demonstrated that the delivery of encapsulated immunomodulators to the cytoplasm of macrophages rendered the cells highly tumoricidal *in vitro* and *in vivo*. Moreover, the repeated intravenous injections of liposomes containing immunomodulators was responsible for the eradication of spontaneous melanoma metastases in the lungs and lymph nodes of syngeneic mice [19,20]. In a recent editorial, Schroit et al. [21] proposed that exploitation of liposome targeting to cells of the RES may enhance therapeutic efficacy against a variety of parasitic, fungal, and bacterial macrophage-associated diseases including schistosomiasis, leishmaniasis, histoplasmosis, cryptococcosis, brucellosis, and salmonellosis. Viral diseases potentially treatable by liposome-encapsulated drugs were not included in this review.

Several groups of viruses productively infect cells of the RES, and virus replication in monocytes–macrophages can be an integral factor in the pathogenesis of certain severe systemic virus infections [22,23]. Macrophages are a primordial defense cell and by virtue of their location throughout the body and capacity to respond to chemotactic stimuli often accumulate early at the sites of many virus infections [24]. Moreover, when activated by a variety of factors, macrophages become selectively cytostatic and cytotoxic for virus-infected cells without damaging normal cells [25–27].

We would like to propose now that the natural targeting of liposomes to mononuclear phagocytes should be exploited to examine whether the selective delivery of antiviral or immunomodulatory agents could be beneficial for the treatment of viral

diseases. In this report we wish to discuss the potential use of liposomes in the treatment of viral diseases from the following perspectives: (a) targeting of liposome-encapsulated immunomodulators to macrophages in order to render these cells cytolytic for virus-infected cells; (b) targeting of liposome-encapsulated antiviral drugs or immunomodulators to macrophages to achieve direct suppression of virus replication within these cells; (c) the advantages, limitations, and future directions for these approaches for therapy.

Targeting of liposome-encapsulated immunomodulators to macrophages in order to render these cells cytolytic for virus-infected cells

The major thrust of current antiviral research is directed toward the development of drugs that selectively block virus replication without causing toxic effects to the host cell. This strategy has led to the development of compounds such as amantadine, an anti-influenza agent that inhibits virus uncoating [28], and acyclovir, an anti-herpes agent that inhibits herpes simplex virus (HSV)-specified DNA polymerase [29]. However, the present lack of control of and treatment for the overwhelming majority of viruses pathogenic to man and animals mandates the search for other therapeutic modes.

Most acute viral infections produce an inflammatory response with characteristic perivascular infiltration of mononuclear cells, the majority of which are mononuclear phagocytes [30]. Macrophages are important components of the host's frontline defense against virus infections, and their strategic location at the portal of entry for most viruses and in the blood and visceral organs facilitates this task. Macrophage accumulation at sites of primary virus infections is enhanced by the release of chemotactic stimuli from foci of virus replication [1]. In viral reinfections, the interaction of virus-coded proteins with sensitized lymphocytes is postulated to trigger the release of soluble mediators, i.e. lymphokines, that are chemotactic to macrophages and can also activate the macrophages for antiviral effects [31]. Activated macrophages acquire the capability of discriminating between virus-infected and normal cells [25–27]. The mechanism by which macrophages select virus-infected from uninfected cells is unknown, although virus-induced changes in the macromolecular constitution of the host cell plasma membranes appear to play a major role [32]. In fact, recent studies with recombinant reoviruses have suggested that the recognition site on reovirus-infected target cells for mouse peritoneal cells is the virus hemagglutinin protein [33]. Collectively, the data suggest that macrophages are an important factor in host defense against viral diseases.

A list of acute viral infections in which selective cytostatic and cytolytic effects of macrophages on virus-infected cells have been demonstrated is shown in Table 1. In addition to these effects, macrophages also restrict the replication of several viruses in infected cell cultures (Table 2). Enhancement of antiviral activity of macrophages can also occur subsequent to their interaction with nonspecific bacterial products or lymphokines [45]. Despite these significant antiviral activities *in vitro*, most attempts at immunomodulation for the prophylaxis and treatment of viral infections *in vivo* by

TABLE 1

Acute viral infections sensitive to macrophage-mediated cytostasis and/or cytolysis

Virus	Reference
Influenza	25, 34
Sendai	25, 34
Reovirus	33
Vaccinia	26
Herpes simplex type 1	26
Herpes simplex type 2	27
Respiratory syncytial	35

TABLE 2

Macrophage-mediated suppression of virus production

Virus	Reference
Encephalomyocarditis	36
Mouse hepatitis	37
Herpes simplex type 1	38
Herpes simplex type 2	39
Vesicular stomatitis	39, 40
Vaccinia	41
Ectromelia	42
Cytomegalovirus	43
Sindbis	30
Influenza	44

administration of nonspecific macrophage activators have met with only marginal success [23]. This may be due in part to the rapid clearance of lymphokines and macrophage activators from the circulation and to the inability to target sufficient quantities of immunomodulators to the macrophages.

Recent studies in our laboratory have demonstrated that macrophages and human peripheral blood monocytes can be activated *in vitro* by a variety of free and liposome-encapsulated substances such as lymphokines containing macrophage-activating factor (MAF), human recombinant gamma interferon, bacterial lipopolysaccharide, or muramyl dipeptide derivatives, to selectively lyse HSV-infected cells without harming uninfected cells [27,46]. Calculations of the internal volume of these liposomes indicated that the total amount of liposome-encapsulated immunomodulators was approximately 800 times lower than the volume of free substances needed to induce macrophage activation to a cytolytic state. Since comparable levels of cytotoxicity against virus-infected cells were observed for free and liposome-encapsulated immune modifiers, these results demonstrated that liposome encapsulation significantly augmented the efficiency of macrophage activation. Control experiments, where macrophages were incubated with liposomes containing culture media, and suspended in

100-fold dilutions of MAF, did not exhibit activation to a cytotoxic state. Thus, the mechanism of macrophage activation via liposome-encapsulated immunomodulators was not simply due to an alteration of macrophage function by liposomes, but required the internalization of the immunomodulatory substance. These results closely agree with the amplification of tumoricidal activity of macrophages by liposomes containing immunomodulators [47,48]. Since intravenously administered liposomes are cleared by macrophages, we evaluated the efficacy of this treatment in a HSV-2 murine model system. Liposomes containing macrophage activators significantly protected mice from a lethal infection with HSV-2, whereas administration of free, unencapsulated immunomodulators produced but marginal effects (Koff, W.C. et al., submitted for publication). The data indicate that the delivery of macrophage activators to cells of the RES via liposomes can significantly modulate the outcome of an acute viral infection.

Targeting of liposome-encapsulated antiviral drugs/immunomodulators to macrophages to achieve direct suppression of virus replication within these cells

Interactions between viruses and macrophages cover a broad spectrum of events ranging from phagocytosis and destruction of virus particles to acute and persistent virus infection [43]. The factors that influence the outcome of virus-macrophage interactions are complex; these include virus type [49], presence or absence of virus-specific antibody [50], availability of viral receptors on macrophages [33], genetic susceptibility [51], age [52-54], immunocompetence of the host [50,55], and stage of differentiation of macrophages in the progression from the unstimulated to the cytolytic phenotype [23].

Table 3 describes the groups of viruses in which replication has been demonstrated to occur within macrophages, along with the corresponding human diseases caused by these viruses. These findings are by no means confined to human viral infections. In Table 4, we list several severe systemic viral diseases of domestic animals where virus replication is also known to occur in macrophages. The economic importance of such diseases has been estimated in billions of dollars [72]. The inability to control these viruses by vaccine or chemotherapy signifies a need to examine other therapeutic approaches.

Since liposomes are targeted naturally to monocytes following intravenous administration, it should be possible to direct antiviral drugs or immunomodulators to viruses replicating within macrophages, and thereby modify the virus infection. Recent studies by Kende et al. [66] support this hypothesis. In those studies, Rift Valley fever virus (RVFV), which replicates in liver macrophages and the central nervous system (CNS), was examined in a murine model system in which small inocula (25 plaque-forming units) causes death 8 days after infection due to liver necrosis and CNS inflammation. The intravenous injection of liposomes containing a lipophilic derivative of muramyl dipeptide (MDP) brought about significant therapeutic effects as measured by mean survival time and percent survival. These effects were observed even in mice treated as late as 5 days after infection [66]. Intravenous administration

TABLE 3

List of viruses that infect human monocytes-macrophages

Genus	Species/serotype	Human disease	Reference
Arenavirus	Junin virus	Argentine hemorrhagic fever	57
Morbillivirus	Measles virus	Measles; subacute sclerosing panencephalitis	58
Vesiculovirus	Vesicular stomatitis virus	Flu-like illness	59
Flavivirus	Dengue virus (4 serotypes)	Dengue fever; dengue hemorrhagic fever	60
	Yellow fever virus	Fever; nephritis; hepatitis	61
Reovirus	Reovirus type 1	None known	33
Herpesvirus	Herpes simplex type 1	Stomatitis; encephalitis disseminated disease	62
	Herpes simplex type 2	Genital disease; disseminated disease	63
	Cytomegalovirus	Disseminated disease; hepatitis; pneumonia	64
	Varicella zoster virus	Varicella; herpes zoster	65
Bunyavirus	Rift Valley fever virus	Disseminated disease; fever	66

TABLE 4

List of animal viruses that infect monocytes-macrophages

Virus	Host	Disease	Reference
Canine distemper	Dogs	Canine distemper	67
Peste des petits ruminants	Goats	Disseminated disease	67
Venezuelan equine encephalomyelitis	Horses	Encephalitis	68
Japanese encephalitis	Swine	Abortion and stillbirth	69
Rift Valley fever	Sheep, cattle	Disseminated disease; fever	66
Equine infectious anemia	Horses	Recurring fever; anemia	70
Caprine arthritis encephalitis	Goats	Leukoencephalomyelitis periarthritis; synovitis	71

of free MDP, even at 100 times the dose administered in liposomes, was not effective. These findings suggest that activation of macrophages by liposomes containing immunomodulators suppresses the replication of viruses in these cells, thereby inhibiting the spread of infection and consequently diminishing the severity of disease. This same principle theoretically could be tested with broad spectrum antiviral agents such as interferon and ribavirin. In fact, the encapsulation of active alpha and gamma interferons in liposomes has already been successfully accomplished *in vitro* [46,73].

Liposome-mediated antiviral therapy: advantages, limitations, and future directions

In this report, we have described the potential use of liposomes as vehicles for the selective delivery of antiviral agents and immunomodulators to monocytes-macrophages for the prophylaxis and therapy of viral diseases. Although this approach is logical and attractive, the field is in its infancy and our expectations need to be realistic. The advantages, limitations and possible future directions of liposome-mediated antiviral therapeutics must now be considered.

Advantages

Liposomes provide an attractive vehicle for the delivery of drugs to macrophages. Because liposomes concentrate in these phagocytic cells, drug concentration in the cytoplasm of macrophages can be manifold higher than in other, nonphagocytic cells. For this reason, the total dose of antiviral drug administered in liposomes could well be several logs less than that used when the drugs are injected in a free (unencapsulated) form. The reduced dose of drugs coupled with selective drug targeting should naturally bring about a significant reduction in systemic or even localized drug-associated toxicity.

Liposomes also provide a vehicle for the delivery of two or more complementary antiviral agents to the same target cell. The presence of more than one antiviral agent, in the desired combinations and ratio, at the site of viral replication in situ could have synergistic antiviral activity. The encapsulation of lymphokines and MDP within the same liposome has been shown to induce synergistic activation of tumoricidal properties in alveolar macrophages [48]. Similar results could well be obtained with combination chemotherapy for the treatment of drug-resistant virus mutants or as a means of lowering the dose of drugs required for antiviral efficacy.

Limitations

The use of liposomes containing immunomodulators for treatment of some viral diseases may be contraindicated. Some viruses reportedly have enhanced replication in 'activated' macrophages [23]. Recent in vitro studies by Hotta and Hotta demonstrate that dengue viruses produce significantly greater titers of virus in activated macrophages than in unstimulated cells [74]. Similarly, alphaviruses [75,76] and coronaviruses [77] replicate with greater efficiency in macrophages treated with a variety of stimulating agents than in resident macrophages. Thus, when virus replication in macrophages is at issue, it is important to analyze the ability of the virus in question to replicate in activated macrophages prior to examining in vivo antiviral therapeutic efficacy of liposome-encapsulated immunopotentiating agents.

The temporal association of the virus-infected cell with the activated macrophage and the kinetics of cytotoxicity may also play a prominent role where therapeutic effects are related to the macrophage mediated lysis of virus-infected cells. Killing of

cells infected with a cytolytic virus before assembly of infectious virus would abort the replication cycle [78]. Even the lysis of cells during virus assembly would most probably result in a reduction of virus and limitation of virus spread. However, lysis of the infected cells just prior to virus-induced lysis could enhance the disease process by speeding up the cytolysis of infected cells and concomitant virus spread. Thus, macrophage-mediated lysis of cells infected by viruses with relatively slow replication cycles (e.g. cytomegalovirus) might be more effective than lysis of cells infected with faster replication cycles (e.g. influenza).

Future directions

Although treatment of macrophage-associated diseases of parasitic and fungal origin by liposome-encapsulated drugs is receiving much attention [21,79], there has been a paucity of information of the potential use of the approach for the treatment of viral diseases. Several important areas of research need to be examined concerning this approach to viral therapeutics. These include determination of the virus groups susceptible to treatment in vitro and in vivo by liposome-encapsulated antivirals/immunopotentiating agents; nature of the phospholipid constitution of the liposomes that produce optimal therapeutic effects; dosage schedules; combination chemotherapy studies; and toxicity studies. In addition, recent findings demonstrate that some persistent and slow viruses such as caprine arthritis encephalitis virus replicate in macrophages [71]. By modifying the internal biochemical nature of macrophages with immunopotentiating agents known to significantly increase lysosomal enzyme and metabolic rates in macrophages [80,81], it might also be possible to modulate these chronic infections. In any event, the selective targeting of liposomes containing antiviral or immunopotentiating agents to phagocytic cells in the body could provide an important new approach for the therapy of several debilitating viral diseases.

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