Polymeric Microspheres As Antigen Delivery Systems

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Vaccination has been considered to be the most effective way to control infectious diseases. Currently, many vaccines used in humans are live-attenuated or killed microorganisms. Polio, mumps, and measles vaccines are live-attenuated. Killed vaccines include cholera and pertussis vaccines. These conventional vaccines, however, suffer from some problems. In the case of live-attenuated vaccines, reversion to virulence is observed in a small but significant number of clinical cases each year. In killed vaccines, due to the possible hazard to employees working with live pathogens, the cost of preparation is high. Killed vaccines also need to be given in multiple doses. Furthermore, both live-attenuated and killed vaccines have possible presence of cellular materials leading to side effects. Moreover, there are diseases such as malaria and hepatitis for which conventional attenuated and killed vaccines are not available because the pathogens cannot be grown in sufficient amounts to allow the classical methods to be used.

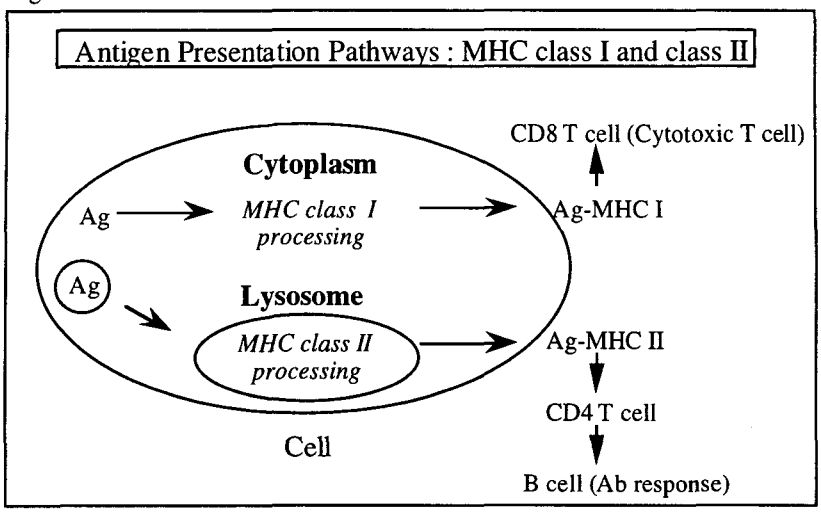
Confronted with the limitations of conventional vaccines, subunit vaccines emerge as a new generation of vaccines. Subunit vaccines are based on either bacterial toxins or purified peptides of antigenic moieties. Subunit vaccines have advantages over live-attenuated and killed vaccines; they are chemically well-defined, can be prepared reproducibly and assayed readily. In addition, advances of biochemical purification and recombinant DNA techniques made it possible to produce pure antigen peptides or proteins in large quantities at low cost.

However, these subunit antigens are in general weak immunogens, and their immunity is limited to an antibody response, possibly due to their instability in vivo and lack of delivery into intracellular sites of antigen processing. For the development of effective subunit vaccines, it is thus essential to employ safe and effective antigen delivery systems that can deliver the antigens into antigen-presenting cells, and further to intracellular target sites.

Intracellular target sites of antigen delivery

The immune system has two major pathways of processing antigens; MHC class I and MHC class II. The MHC class II pathway stimulates antibody-mediated humoral immunity whereas MHC class I pathway primes for cytotoxic T lymphocyte (CTL) immunity. The importance of the intracellular antigen delivery sites lies in that the subcellular locations of antigens determine whether the antigens will be processed by MHC class I or class II pathway (Fig. 1).

Fig. 1.



i) Lysosomes: the target site for MHC class II antigen processing

When antigens are delivered into lysosomes, they are processed by MHC class II pathway, presented on the cell surface with MHC class II molecules, and stimulate CD4 T cells. CD4 T cells then activate B cells, and enhance antibody response against the antigens.

ii) Cytoplasm: the target site for MHC class I antigen processing

MHC class I pathway is present in cytoplasm, and involved in the processing of antigens delivered into cytoplasm. Antigens in cytoplasm are processed by proteasomes, transported into Golgi, presented on the cell surface with MHC class I molecules, and prime for cytotoxic CD8 T cells.

In immunity, enhanced antibody levels would be helpful in protecting the body from toxins and pathogens in the blood stream. However, for virus and intracellular bacteria residing in the cells, antibody can not effectively attack those pathogens due to limited accessibility. CTL are a powerful weapon killing such infected cells. CTL immunity, however, has been hard to achieve by killed vaccines and subunit vaccines since these vaccines usually target to lysosomes, not to cytoplasm.

Thus, "ideal" delivery systems for viral and intracellular bacterial antigens should deliver antigens not only to lysosomes (MHC class II pathway) for antibody response, but also to cytoplasm (MHC class I pathway) for CTL immunity.

Polymeric microspheres: antigen delivery systems boosting both humoral and cytotoxic T lymphocyte immunity

There are several requirements of ideal antigen delivery systems. Antigen delivery systems need to be non-pathogenic, non-toxic, and easy and inexpensive to manufacture on a large scale. In addition, they should give long-lasting immunity by a single dose preferably via oral route. Moreover, they need to

deliver antigens effectively to antigen-presenting cells such as macrophages, and further to intracellular target sites of antigen processing, lysosomes and cytoplasm. However, the methods of delivering antigens into cytoplasm have been considered to be difficult.

Among various antigen delivery systems such as immunostimulating complexes, liposomes, and polymeric microspheres, polymeric microspheres have drawn more attention since they possess several characteristics of "ideal" antigen delivery systems. First, the controlled or pulsed release of antigens from microspheres can minimize the vaccination frequency. Second, the relative stability in the gut and the uptake into gut-associated lymphoid tissue allow the design of polymeric microspheres as an oral antigen delivery system. Above all, very recent findings suggest that polymeric microspheres might satisfy the important but hard-to-achieve requirement of ideal antigen delivery system, the subcellular delivery of antigens to both lysosomes and cytoplasm.

i) Lysosomal antigen delivery and humoral immunity (antibody response)

Several reports showed the potential of polymeric microspheres as antigen delivery systems providing long-lasting humoral immunity. Subcutaneous administration of biodegradable poly(DL-lactic-co-glycolic acid) (PLGA) microspheres containing staphylococcal enterotoxin B (SEB) could enhance the plasma level of toxin-neutralizing antibodies at least for 110 days after a single injection (Fig. 2). Purified enterotoxigenic E. coli colonization factor antigen (CFA) entrapped in PLGA microspheres provided significantly higher antibody response over free antigen at least for 120 days after oral immunization (Fig. 3).

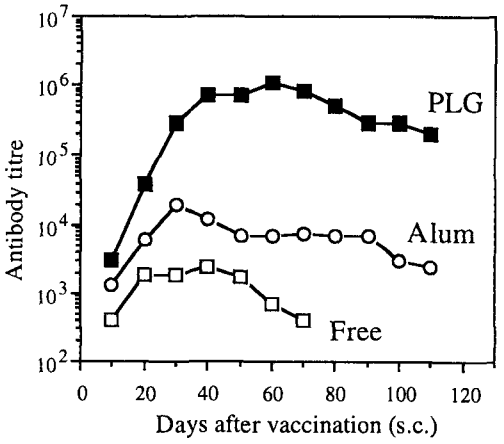


Fig. 2. Antibody response to subcutaneouly administered SEB antigen.

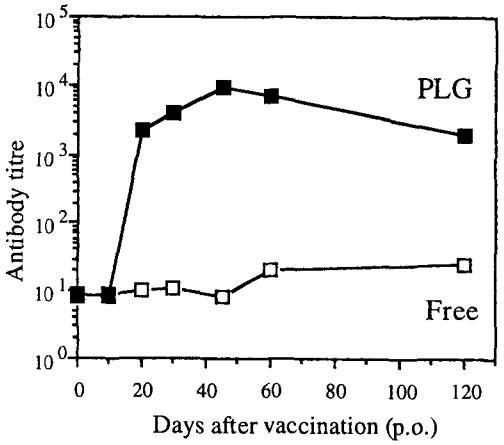


Fig. 3. Antibody response to orally administered CFA antigen.

Regarding the intracellular delivery sites, various polymeric microsphere-containing phagosomes progressed to lysosomes within 20 min after phagocytosis.

The kinetic of lysosome targeting was not affected by chemical nature and surface properties of microspheres. As polymeric microspheres, biodegradable polycaprolactone microspheres (PCL) and polystyrene microspheres (PS), amine-derivatized PS (amine-PS), polyethylene glycol-conjugated PS (PEG-PS) were used. Various lysosome markers including fluorescein dextran, lamp-1 (lysosomal membrane glycoprotein), and cathepsin-D (lysosomal protease) showed the similar kinetics of intracellular trafficking of polymeric microspheres to lysosomes in macrophages (Fig. 4).

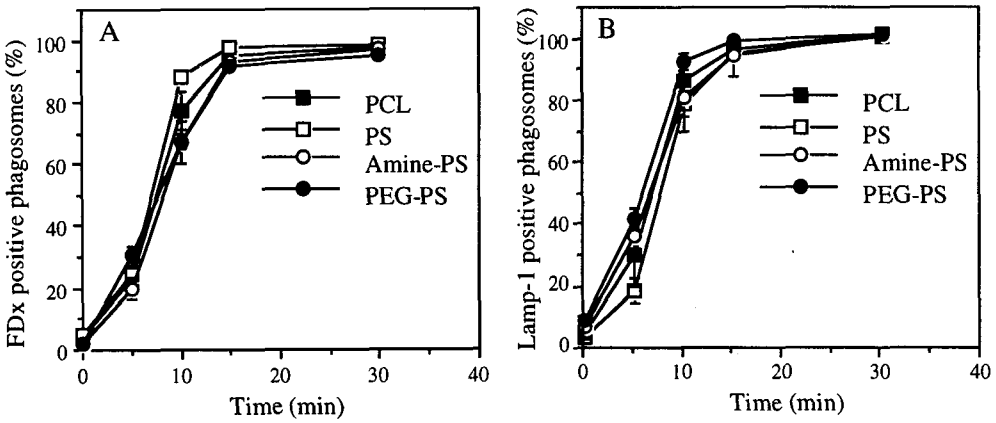


Fig. 4. Kinetics of microsphere-containing phagosome progression to lysosomes. As lysosome markers, fluorescein dextran (A) and lamp-1 (B) were used.

These results indicate that polymeric microspheres could deliver their antigens into lysosomes and stimulate humoral immunity over prolonged period.

ii) Cytoplasmic antigen delivery and cytotoxic T lymphocyte immunity

As mentioned above, CTL immunity is the effector mechanism for killing infected cells. Inducing CTL immunity by cytoplasmic antigen delivery is thus a crucial issue in designing effective vaccines against viral and intracellular bacterial infections. However, few studies have tested the potential of polymeric microspheres as antigen delivery systems inducing CTL immunity, although substantial amounts of research proved polymeric microspheres as antigen delivery systems capable of enhancing humoral immunity. One reason for the lack of reports on CTL immunity is that most antigens delivered by polymeric microspheres were bacterial toxins for which humoral immunity might be enough to give protection. Another reason is that the antigens carried to lysosomes by polymeric microspheres have been dogmatically believed to remain only in lysosomes, while being processed by MHC class II pathway.

Against the dogma, very recently, it was reported that particulate antigens based on magnetic particles or latex microspheres could induce CTL immunity. However, it has not been known whether CTL immunity, elicited by a few type of

particles, can be efficiently generated in pharmaceutically more meaningful particulate antigen delivery systems. Furthermore, it has not been clear whether the primed CTL immunity by particles is via the delivery of antigens into cytoplasm and the classical MHC class I pathway.

We thus studied the efficiency and duration of CTL immunity of a model antigen, ovalbumin (OVA), given by various delivery systems such as sheep red blood cells (SRBC), PS, and biodegradable PCL microspheres. Among these particles, PCL showed the highest efficiency of CTL immunity (Fig. 5). PCL also offered the most prolonged CTL immunity (Fig. 6).

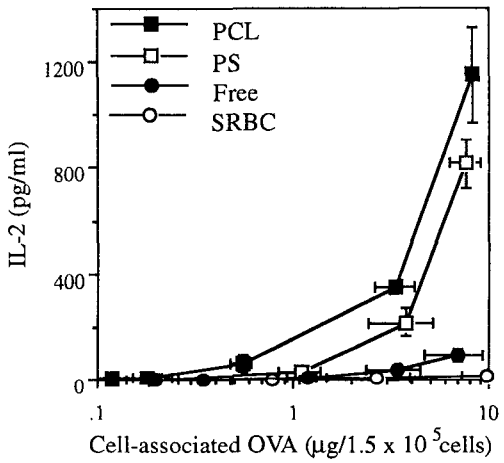


Fig. 5. Efficiency of CD8 T cell stimulation.

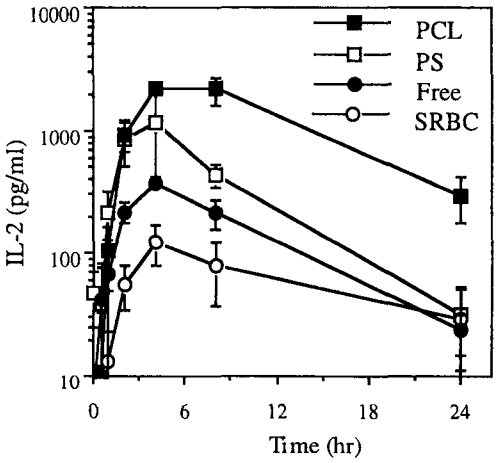


Fig. 6. Duration of CD8 T cell stimulation.

A fluorescence probe technique, based on fluorescein biotin-streptavidin binding, provided morphological evidence that polymeric microspheres could deliver antigens into cytoplasm. PCL delivered antigens into cytoplasm, whereas SRBC which did not show show significant CTL immunity (Fig. 5), did not deliver antigens into cytoplasm. To test whether the conventional MHC class I pathway is involved in processing antigens delivered into cytoplasm, inhibitors of the pathways were used (Fig. 7). Proteasome inhibitors such as lactacystin and MG 132 (Fig. 8), and an inhibitor of ER-to-Golgi transport (brefeldin A) blocked CTL immunity, suggesting that antigens delivered into cytoplasm by polymeric microspheres were processed by the classical MHC class I pathway. These results indicate that polymeric microspheres could deliver antigens into cytoplasm and prime for CTL immunity via the MHC class I pathway.

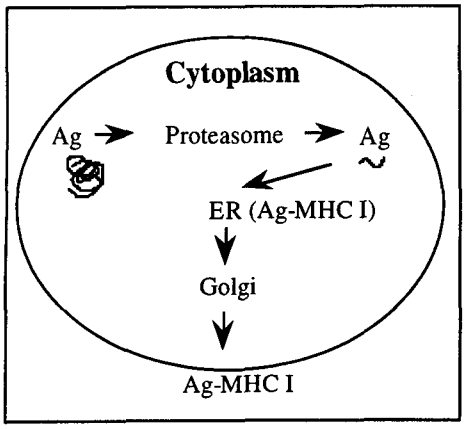


Fig. 7. MHC class I antigen presentation pathway.

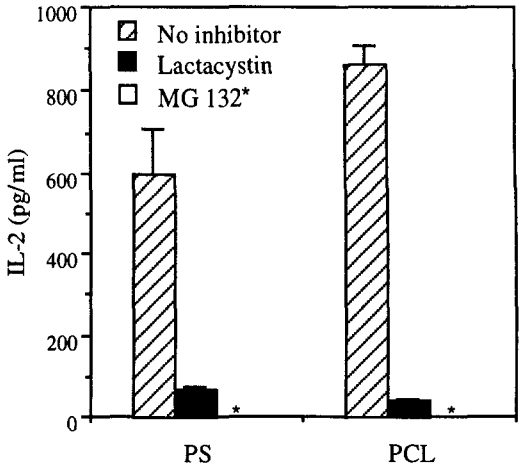


Fig. 8. Effect of proteasome inhibitors on CD8 T cell stimulation.

Conclusion

Our results show that biodegradable polymeric microspheres could deliver antigens into both lysosomes and cytoplasm, the target sites for MHC class II and MHC class I antigen processing, respectively. The high efficiency and prolonged duration of humoral and CTL immunity of biodegradable polymeric microspheres suggest the potential of these microspheres as effective antigen delivery systems for viral and intracellular bacterial infections.