Polymer and Material Sciences and Engineering 66, 493-494(1992)

BIODEGRADABLE POLYMERS FOR THE CONTROLLED DELIVERY OF VACCINES.

E. Schmitt, D.R. Flanagan, R.J. Linhardt, Division of Pharmaceutics and Division of Medicinal and Natural Products Chemistry, College of Pharmacy, University of Iowa, Iowa City, Iowa 52242

Introduction

Biodegradable polymers have been used for a variety of medical applications including the controlled release of drugs and biologicals [1]. One potential application of biodegradable polymers is the controlled delivery of vaccines. The advantages of using these systems are first that if properly designed, the release kinetics can obviate the need for repeated booster regimens which would be a substantial advantage in third world countries where compliance may make it difficult to administer repeated injections. Secondly, many new vaccines synthesized using recombinant techniques are insufficiently immunogenic alone to be successful thus requiring the use of adjuvants. Our laboratory has shown that a wide range of potential *in vitro* release profiles may be achieved by careful selection of the polymer and formulation method [2]. By modifying the release kinetics or incorporating both vaccines and adjuvants into biodegradable polymers the immunogenicity of many new vaccines may be greatly improved.

Initial studies on polymers for the sustained release of antigen.

As early as 1976, Chang [3] described the use of poly(lactic acid) for the microencapsulation of vaccines and antigens but did not study the release properties or immunogenicity of these formulations. Preis and Langer [4] later showed that the sustained release of bovine serum albumin, ribonuclease-A and bovine γ-globulin from ethylene-vinyl acetate matrices resulted in sustained antibody levels in mice for 25 weeks. The antibody levels in mice implanted with a single BSA/polymer pellet (50 μg BSA) were comparable with those found in mice given a primary injection of BSA (50 μg) in complete Freund's adjuvant (CFA) followed by an identical booster regimen seven days later (100 μg total BSA) (Figure 1). These results showed that the sustained release of antigens from polymeric matrices could indeed elicit a substantial immune response with a single treatment. The polymer used however did not degrade *in vivo* and thus would require surgical removal after some time making it less convenient than traditional inoculations. These experiments did however show that the sustained release of vaccines and adjuvants from polymeric matrices was a viable route for single-step immunization.

Synthetic biodegradable polymers as delivery systems.

Kohn, et al. [5] studied an antigen delivery system based on poly(CTTH-iminocarbonate) (IUPAC nomenclature: poly[oxyimidocarbonyloxy-p-phenylene[2-(hexyloxycarbonyl)ethylene]imino[2-[1-(benzyloxy)formamido]-1-oxotrimethylene]-p-phenylene]). This is an interesting polymer because its primary degradation product, N-benzyloxycarbonyl-L-tyrosyl-L-tyrosine hexyl ester (CTTH) was found to be as potent an adjuvant as Freund's complete and muramyl dipeptide (MDP). *In vitro* release profiles appeared to follow a square-root of time dependence with most of the antigen released within 25 days. *In vivo* studies conducted in mice showed anti-BSA antibody titers after 56 weeks were over twice as high in animals given a single injection of the poly(CTTH-iminocarbonate) formulation as compared to animals given BSA at 0 and 4 weeks.
Within the last year, the poly and copoly(esters) of lactic acid and glycolic acid have been shown to be promising materials for vaccine and antigen delivery systems. The advantages of using these polymers is that they have already been used in humans for many years as surgical sutures and as such, their approval by regulatory agencies should be easier to obtain. O'Hagan et al. [6] studied the immunogenic response of mice to ovalbumin entrapped in poly(D,L-lactide-co-glycolide, 50:50) by two routes of immunization, subcutaneous (*s.c.*) and intraperitoneal injection (*i.p.*). Antibody (IgG) levels in response to injections of soluble ovalbumin, ovalbumin emulsified in CFA and ovalbumin entrapped in poly(ester) microcapsules were measured. Both primary and secondary responses were measured. Ovalbumin (OVA) is a rather poor immunogen thus the results of this study are useful in assessing the adjuvant effect of microcapsule delivery system. The results for the primary immune response showed that after single *i.p.* injections, the mice given encapsulated OVA had a significantly greater IgG titer at 10 weeks post injection. The differences among the formulations were not significant for the secondary response. In a related study, Eldridge et al. [7] found that poly(D,L-lactide-co-glycolide) microspheres represented a potent adjuvant system for *Staphylococcal* enterotoxin B (SEB) capable of inducing both circulating and mucosal immunity. Eldridge et al. [8] also found that orally administered poly(D,L-lactide-co-glycolide) 1-10 μm microspheres containing SEB were specifically taken up into the Peyer's patch lymphoid tissue of the gut. Furthermore, SEB containing microspheres induced circulating anti-SEB antibodies and a secretory IgA anti-SEB response in saliva and gut fluid, while soluble SEB did not. In yet another study, Eldridge et al. [9] evaluated the immune response of mice given SEB containing poly(D,L-lactide-co-glycolide) microspheres. The results showed that the kinetics, magnitude and duration of the immune response to encapsulated SEB were similar to those obtained after an equivalent dose emulsified in CFA (Figure 2). The microspheres had an additional benefit of not inducing inflammation and granulomata seen in CFA. Antigen containing microspheres 1 to 10 μm in diameter exhibited stronger adjuvant activity than those > 10 μm suggesting that uptake of the microspheres by macrophages is an important factor in the adjuvant activity.

Future Approaches

In the future the mechanism by which vaccines, encapsulated in biodegradable polymers induce immunization requires study. This may suggest the use of alternative types of biodegradable polymers having adjuvanting activities. Another consideration is that ideal release kinetics may not be zero-order or matrix-controlled but instead may be pulsatile in nature mimicking standard immunization protocols. Only continued research can answer these important questions.

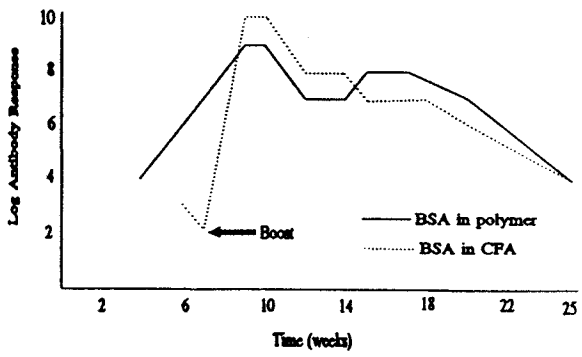

Figure 1: Antibody response to BSA in CFA given in two doses as compared to sustained release from a polymeric matrix. (Adapted from reference 4)

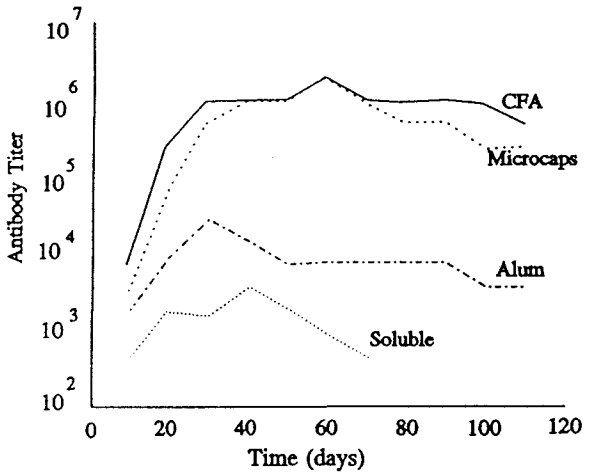

Figure 2: Antibody response to SEB toxin formulationss. (Adapted from reference 9.)

Acknowledgment

Mr. Eric A. Schmitt gratefully acknowledges the financial support of Bristol-Myers Squibb and the American Foundation For Pharmaceutical Education.

References

1. R.J. Linhardt, Biodegradable polymers for the controlled release of drugs. In: M. Rosoff (Ed.), Controlled Release of Drugs: Polymers and Aggregate Systems, VCH Publishers, New York, pp. 53-95 (1989).

2. H.T. Wang, E. Schmitt, D.R. Flanagan and R.J. Linhardt, Influence of formulation methods on the in vitro controlled release of protein from poly(ester) microspheres. *J. Controlled Release*, 17, 23-32 (1991).

3. T.M.S. Chang, Biodegradable semipermeable microcapsules containing enzymes, hormones, vaccines, and other biologicals. *J. Bioeng.*, 1, 25-32 (1976).

4. R.S. Langer and I. Preis, A single-step immunization by sustained antigen release. *J. Immunol. Methods*, 28, 193-197 (1979).

5. J. Kohn, S.M. Niemi, E.C. Albert, J.C. Murphy, R. Langer and J.G. Fox, Single-step immunization using a controlled release, biodegradable polymer with sustained adjuvant activity. *J. Immunol. Methods*, 95, 31-31 (1986).

6. D.T. O'Hagan, D.R., J.P. McGee, H. Jeffery, M.C. Davies, P. Williams, S.S. Davis and S.J. Challacombe. Biodegradable microparticles as controlled release antigen delivery systems. *Immunology*, 73, 239-242 (1991).

7. J.H. Eldridge, J.K. Staas, J.A. Meulbroek, J.R. McGhee, T.R. Tice and R.M. Gilley. Biodegradable microspheres as a vaccine delivery system. *Molecular Immunol.*, 28, 287-294 (1991).

8. J.H. Eldridge, R.M. Gilley, J.K. Staas. Z. Moldoveanu, J.A. Meulbroek and T.R. Tice. Biodegradable micro-spheres: vaccine delivery system for oral immunization. *Curr. Top. Microbiol. Immun.*, 146, 59-66 (1989).

9. J.H. Eldridge, J.K. Staas, J.A. Meulbroek, T.R. Tice and R.M. Gilley. Biodegradable and biocompatible poly(DL-lactide-co-glycolide) microspheres as an adjuvant for staphylococcal enterotoxin B toxoid which enhances the level of toxin-neutralizing antibodies. *Infect. Immun.*, 59, 2978-2986 (1991).