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Controlled release microparticles for oral immunization

D.T. O'Hagan [a,*], D. Rahman [b], H. Jeffery [a,c], S. Sharif [a], S.J. Challacombe [b]

[a] *Department of Pharmaceutical Sciences, University of Nottingham, Nottingham, UK*
[b] *UMDS, Department of Oral Medicine and Pathology, Guy's Hospital, London, UK*
[c] *Current address: Biotech Australia Pty Ltd, 28 Barcoo St., Roseville, NSW 2069, Australia*

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Abstract

Microparticles with entrapped ovalbumin (OVA) were prepared using two different poly(lactide-co-glycolide) polymers (Resomers RG 506 and 755) with different rates of degradation and were orally administered to two groups of mice. Both groups showed enhanced serum IgG and salivary IgA antibody responses in comparison to a group of mice immunized with soluble OVA, but the level of responses induced depended on the polymer. The more rapidly degrading polymer (RG 506) was most effective for the induction of high levels of salivary IgA antibodies, while the more slowly degrading polymer (RG 755) was most effective for the induction of serum IgG antibodies.

Key words: Biodegradable microparticles; Oral immunization; Poly(lactide-co-glycolide); Controlled release

1. Introduction

During the last decade, significant advances in peptide synthesis and molecular biology have made available a wide range of antigens, that may be used for the development of novel vaccines against a range of infectious diseases (Brown, 1990). Nevertheless, these purified peptide and protein antigens are often poorly immunogenic and require formulation with potent adjuvants or delivery systems to enhance their immunogenicity. Consequently, in recent years, there has been a considerable expansion in research into adjuvants and antigen delivery systems (Epstein et al., 1990), and many candidate delivery systems have arisen. These candidate antigen delivery systems include both live and non-replicating carriers, but none has yet advanced to large scale trials in humans. One specific delivery system which holds considerable future promise, comprises polymeric micropartiacles with entrapped antigens (Eldridge et al., 1991; O'Hagan et al., 1991a,b). Microparticles are particularly attractive for immunization in the developing world because of their controlled release characteristics, which enables them to induce long-term antibody responses following a single immunization (O'Hagan et al., 1993).

* Corresponding author: Director of Vaccine Formulation, United Biomedical, Inc., 25 Davids Drive, Hauppauge, NY 11788, U.S.A.

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Nevertheless, ideally, new vaccines should be administered by the oral route. The advantages of oral immunization include easier administration, reduced side-effects and the potential for almost unlimited frequency of boosting, without the need for trained personnel. Oral vaccines would also be safer to administer, since the skin would not be breached during immunization. Moreover, avoiding the use of needles during administration would eliminate the possible risks of contamination and cross-infection due to re-use. In addition, the purchase cost of the needles would be eliminated. Furthermore, a vaccine for oral administration should also be cheaper to manufacture. Vaccines are already the most efficient and cost effective means for disease prevention, but only 12% of the current costs for vaccination is the vaccine itself (Aguado and Lambert, 1992). Since microparaticles are taken up into the Peyer's patches following oral administration (O'Hagan, 1990) they may be used for oral immunization (O'Hagan et al., 1989; Eldridge et al., 1990; Challacombe et al., 1992). Consequently, microparaticles hold considerable promise for the future development of new and improved oral vaccines.

In the present study, earlier observations on the ability of biodegradable poly(lactide-co-glycolide) (PLG) microparticles to induce immunity following oral immunization (Challacombe et al., 1992), were extended by using two different polymers with different rates of degadation to prepare microparticles with an entrapped model antigen, ovalbumin (OVA). The objective of the present study was to determine if microparticles with different antigen release rates would induce different antibody responses following oral immunization.

2. Material and methods

2.1. Animals

Male BALB/c mice (Olac Ltd, Cirencester) aged 6–8 weeks and weighing about 25 g were used and were maintained on a normal mouse diet throughout the studies.

2.2. Microparticle preparation

Micropartaicles with entrapped OVA were prepared using PLG polymers obtained from Boehringer Ingelheim KG (Ingelheim, Germany). The two polymers used (Resomers RG 506 and RG 755) respectively had co-polymer compositions of 50:50 and 75:25 lactide/glycolide, and molecular masses of 84 and 83 kDa, as determined by gel permeation chromatography (O'Hagan et al., 1994). The microparticles were prepared using a solvent evaporation technique as previously described (Jeffery et al., 1993). Briefly, a solution of each polymer in dichloromethane (HPLC grade, May and Baker, Dagenham) was emulsified together with a solution of OVA in double-distilled water using a Silverson homogeniser (Silverson Machines Ltd, Chesham, Bucks) to produce a w/o emulsion. This emulsion was then added to a solution of polyvinyl alcohol (PVA) (88% hydrolysed, Aldrich Chemical Co., Poole) and homogenised to produce a stable w/o/w emulsion. The emulsion was then stirred overnight at ambient temperature and pressure to allow solvent evaporation to proceed, with resultant microparticle formation.

Following preparation, the microparticles were collected by centrifugation, washed three times to remove non-entrapped OVA and freeze-dried. The volume mean diameter of the microparticles was determined by laser diffractometry using a Malvern Laser sizer 2600D and is quoted in the text in μm.

2.3. Determination of OVA entrapment

The amount of OVA in the microparticles was determined in a bicinchoninic acid (BCA) protein assay (Sigma) following disruption of the microparticles and extraction of the entrapped antigen using an established method (Eldridge et al., 1991).

2.4. Immunization protocols

To minimize the number of experimental variables, the microparticles prepared from the two different polymers (RG 506 and 755) were only

used for secondary immunization. For primary immunization, two groups of mice each received OVA entrapped in microparticles prepared from polymer RG 506. Immediately before administration, the required dose of microparticles was weighed and resuspended in physiological saline (0.5 ml).

Three groups of 10 mice each received primary immunization with 1 mg OVA by gastric intubation on three consecutive days, either in saline (one group), or entrapped in microparticles (two groups) prepared from RG 506 (2.1 μm). 4 weeks after the primary immunizations, the three groups of animals were boosted with the same dose of OVA in saline, or in microparticles prepared from polymers RG 506 (2.2 μm) or RG 755 (1.6 μm).

2.5. Collection of blood and salivary samples

Blood samples were collected from the tail veins 4, 8 and 12 weeks after primary immunizations. Salivary samples were collected at 2 weekly intervals from week 1, following stimulation by intraperitoneal injection of pilocarpine (0.5% w/v) as previously described (Challacombe et al., 1992)

2.6. Assay of antibody units

The levels of IgG antibodies in the sera of immunized animals were measured in an ELISA as previously described (O'Hagan et al., 1991b). The levels of sera and salivary IgA antibodies were also measured in an ELISA as previously described (Challacombe et al., 1992). The value for each of four dilutions of the serum or saliva samples falling in the standard curve was calculated and the value for the sample taken as the mean of the four separate dilutions. The results are shown as mean $\pm$ S.E. for each group of mice.

2.7. Statistics

An unpaired Student's t-test was used to compare the means at the different sample times and to assess statistical significance. Results were considered statistically significant if $p < 0.05$.

3. Results

3.1. Entrapment of OVA

The microparticles (RG 506) used for primary immunization contained 6.5% w/w entrapped OVA. For the secondary immunizations, the microparticles contained 5.6% w/w OVA (RG 506) and 4.9% w/w OVA (RG 755). The degradation rates of the two polymers, RG 506 and RG 755, and the rate of release of entrapped OVA from microparticles prepared from these polymers has been reported previously (O'Hagan et al., 1994).

3.2. Salivary IgA antibody responses

From week 5 onwards, 1 week after secondary immunization, both groups of mice given OVA entrapped in microparticles showed significantly enhanced salivary IgA antibody responses in comparison to the soluble group. At week 7, the salivary IgA response to microparticles prepared from RG 506 was significantly enhanced in comparison to the response to microparticles prepared form RG 755 (Fig. 1).

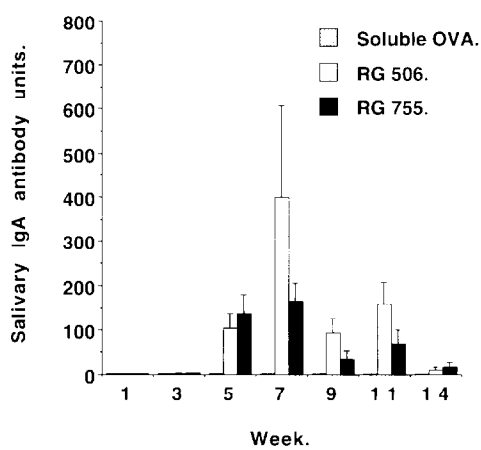

Fig. 1. Salivary IgA antibody responses following oral immunization with OVA in saline, or entrapped in poly(lactide-co-glycolide) microparticles prepared from polymer RG 506. Booster immunizations of soluble OVA and OVA entrapped in microparticles prepared from polymer RG 506 or RG 755 were administered at week 4.

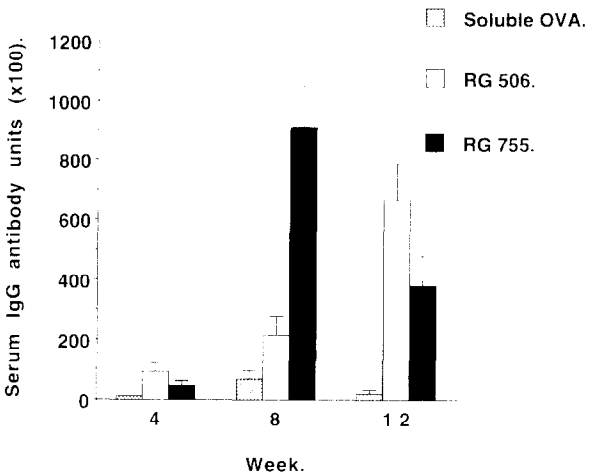

Fig. 2. Serum IgG antibody responses following oral immunization with OVA in saline, or entrapped in poly(lactide-co-glycolide) microparticles prepared from polymer RG 506. Booster immunizations of soluble OVA and OVA entrapped in microparticles prepared from polymer RG 506 or RG 755 were administered at week 4.

3.3. Serum IgG antibody responses

Throughout the study, the serum IgG responses to OVA entrapped in microparticles were significantly enhanced in comparison to the response to soluble OVA. Moreover, at week 8, the response to microparticles prepared from RG 755 was significantly enhanced in comparison to the response to microparticles prepared from RG 506 (Fig. 2).

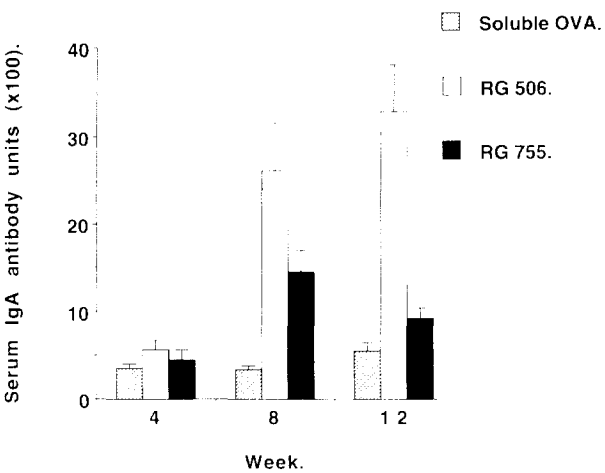

Fig. 3. Serum IgA antibody responses following oral immunization with OVA in saline, or entrapped in poly(lactide-co-glycolide) microparticles prepared from polymer RG 506. Booster immunizations of soluble OVA and OVA entrapped in microparticles prepared from polymer RG 506 or RG 755 were administered at week 4.

3.4. Serum IgA antibody responses

At weeks 8 and 12, the serum IgA responses to microparticles prepared from RG 506 were significantly enhanced in comparison to microparticles prepared from RG 755 and in comparison to soluble OVA (Fig. 3).

4. Discussion

Previous studies had shown that the rate of release of OVA from PLG microparticles in vitro was dependent on the polymer molecular mass, the co-polymer composition and the loading level (O'Hagan et al., 1994). In general, the rate of release of OVA was delayed as the molecular mass increased, the ratio of lactide/glycolide increased and the loading level decreased (O'Hagan et al., 1994). Furthermore, it was shown that for comparable loading levels, the rate of release of OVA from microparticles prepared from RG 755 was slower than that from microparticles prepared from RG 506 (O'Hagan et al., 1994). The microparticles prepared from RG 755 which were used for oral immunization in the current study had a lower loading level of OVA (4.5%), than those prepared from RG 506 (5.6%). Taken together, these observations indicate that the rate of release of OVA from the microparticles used for oral immunization would be slower for the goup immunized with the microparticles prepared from polymer RG 755. Unfortunately, there was not enough of the microparticles remaining from these batches following oral immunization to determine directly the OVA release profiles in vitro. Nevertheless, preliminary observations indicated that the rate of release of OVA from RG 755 microparticles was indeed slower. Moreover, the data reported here from the in vivo studies indicate that different release profiles were

achieved with the two batches of microparticles prepared from polymers RG 506 and RG 755.

Two clear conclusions may be drawn from these studies. The first conclusion allows confirmation of our previous observation that microparticles are capable of inducing enhanced secretory and systemic antibody responses to entrapped OVA following oral immunization (Challacombe et al., 1992). In agreement with our previous study, primary immunization did not induce an enhanced salivary IgA antibody response, but secondary immunzation did. In the previous study, the salivary IgA response peaked at week 6 and optimally was 50 times greater than the response to soluble OVA. In the present study, using a comparable formulation (RG 506), a similiar response was obtained. As with the previous study, the salivary response fell away rapidly. However, in the present study, there was some evidence of a second peak at week 11, which may have resulted from controlled release of OVA from the microparticles. As in the previous study, the serum IgG response to microparticles was significantly enhanced at 4 and 8 weeks after primary immunization. In addition, the longer duration of the present study allowed us to observe that the serum IgG response to microparticles prepared from RG 506 was still rising at week 12. In general, the serum IgA antibody response showed the same profile as IgA in the saliva. The induction of secretory and systemic antibody responses to an antigen that normally induces tolerance following oral administration (Challacombe and Tomasi, 1980) is a very significant and encouraging finding. These observations serve to underline the potential of microparticles as an oral antigen delivery system.

The second conclusion that can be drawn from the current study is that the rate of release of entrapped OVA from microparticles following their uptake into the Peyer's patches has an effect on the antibody responses induced. The microparticles prepared from the more rapidly degrading polymer (RG 506) induced a salivary IgA response that was significantly enhanced in comparison to the more slowly degrading polymer (RG 755). One possible explanation for this finding may be that it is necessary to rapidly deliver large amounts of antigen into the antigen-presenting cells in the Peyer's patches in order to induce high levels of secretory IgA antibodies. Hence, microparticles prepared from RG 506 may have been more successful than those prepared from RG 755 at inducing secretory IgA because the release of entrapped antigen was more rapid following uptake into the Peyer's patches.

The serum IgG antibody responses to orally administered microparticles showed the opposite profile to what might have been predicted in advance of the study. Microparticles prepared from RG 755 showed a rapid serum response and those prepared from RG 506 showed a more delayed response. Nevertheless, the serum responses obtained may possibly be explained with reference to the migratory patterns of microparticles following their uptake into the Peyer's patches. A previous study investigated the fate of similiarly sized PLG microparticles following their uptake into Peyer's patches (Eldridge et al., 1990). The numbers of particles in the Peyer's patches peaked at day 4 after administration and subsequently declined as the particles were transported away in the mesenteric lymph. Similiar observations have also been reported in recent studies undertaken at Nottingham (Jenkins et al., 1994). These observations may help to explain why the microparticles prepared from RG 755 were more effective for the induction of high levels of serum IgG antibodies at week 8 than the microparticles prepared from RG 506. Delayed release of OVA from microparticles prepared from RG 755 may have allowed relatively large amounts of antigen to be delivered to the lymph nodes and the spleen following transport of the microparticles to these sites (O'Hagan, 1990), while the majority of OVA from the RG 506 microparticles may have been released in the environment of the Peyer's patches and consequently, would induce a secretory IgA response.

The release profiles of proteins from PLG microparticles are complex and release often occurs in three distinct phases (Hora et al., 1990a,b; Cohen et al., 1991; O'Hagan et al., 1994). These three phases represent the initial 'burst' release of surface located and poorly entrapped protein, followed by a period of minimal release while

polymer degradation occurs and a third phase of degradation-dependent antigen release, which continues until the microparticles are depleted of protein. Thus, it is difficult to predict with any certainty exactly at which physiological site (Peyer's patches, lymph nodes, spleen, etc.) the OVA was released from the microparticles following their uptake. Moreover, it is also difficult to predict which phase of the OVA release profile is actually responsible for the induction of the different immune responses observed. Nevertheless, the significant conclusion remains that the rate of release of antigen from microparticles after their uptake into Peyer's patches affects the antibody responses induced. Controlled release microparticles may ultimately be exploited to induce a desired profile of antibody responses, including both serum and secretory responses, following oral immunization.

In the present study, the batches of microparticles prepared from different polymers were administered to different groups of mice. However, different batches of microparticles with varying release characteristics may be combined and administered simultaneously as a single dose. Such studies have already been undertaken in mice and an immune response lasting at least 1 year was induced from a single subcutaneous dose of microparticles (O'Hagan et al., 1993). Nevertheless, it is clear from the present study that much still needs to be learned about the secretory and systemic antibody responses to antigens presented orally in microparticles.

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