INFECTION AND IMMUNITY, Mar. 1997, p. 853–857 Vol. 65, No. 3
0019-9567/97/$04.00+0

Protective Immunity against *Salmonella typhimurium* Elicited in Mice by Oral Vaccination with Phosphorylcholine Encapsulated in Poly(DL-Lactide-Co-Glycolide) Microspheres

KHADIJA ALLAOUI-ATTARKI,[1] SOPHIE PECQUET,[1]† ELIAS FATTAL,[2] SYLVINE TROLLÉ,[1] ELISABETH CHACHATY,[1] PATRICK COUVREUR,[2] AND ANTOINE ANDREMONT[2,3]*

Service de Microbiologie Médicale, Institut Gustave-Roussy, 94800 Villejuif,[1] Laboratoire de Pharmacie Galénique, URA-CNRS 1218, Faculté de Pharmacie, 92296 Châtenay-Malabry Cedex,[2] and Laboratoire de Bactériologie, Faculté de Médecine Xavier-Bichat, 75018 Paris,[3] France

Received 16 January 1996/Returned for modification 16 April 1996/Accepted 6 December 1996

Encapsulation of vaccines in biodegradable microspheres provides excellent mucosal immunogens with a high potential for immunization against bacterial infections. We tested the protective immunity elicited by intragastric vaccination with phosphorylcholine (PC) encapsulated in poly(DL-lactide-co-glycolide) (DL-PLG) microspheres against *Salmonella typhimurium* in a mouse model of invasive intestinal infection. We chose PC as the antigen because it was found to elicit an immune response after intestinal exposure of mice to PC-bearing *S. typhimurium* and because anti-PC immunity protects mice against *Streptococcus pneumoniae*, another PC-bearing microorganism. Mice were primed intragastrically on days 1, 2, and 3 and boosted on days 28, 29, and 30 with PC (280 μg) coupled to porcine thyroglobulin (PC-thyr) encapsulated in DL-PLG microspheres, free PC-thyr, or blank microspheres. A significant rise in anti-PC immunoglobulin A (IgA) titers, as measured by an enzyme-linked immunosorbent assay, was observed in the intestinal secretions after immunization with PC-loaded microspheres, compared to the titers of mice immunized with free PC-thyr or blank microspheres. This antibody response peaked 14 days after the last boost and correlated with a highly significant resistance to oral challenge by *S. typhimurium* C5 ($P < 10^{-3}$). Control mice were primed intraperitoneally on day 1 with 15 μg of PC in complete Freund's adjuvant and boosted on days 10, 14, and 20 with the same dose without adjuvant but via the same route. In these mice, the levels of anti-PC IgA in intestinal secretions were equivalent to those of the mice intragastrically immunized with PC-loaded microspheres, but protection was significantly weaker, suggesting that either the IgAs were not functional or that other immune mechanisms are important in protection. Taken together, our results highlight the potential of antigen encapsulation in DL-PLG microspheres for eliciting protective immunity against invasive intestinal bacterial diseases and suggest that a similar strategy could be used against diseases caused by other PC-bearing microorganisms.

The vaccines available to date target protection against single specific diseases. Induction of immunity to multiple infections is based on combining vaccinal antigens and multiplying injections, thus complicating vaccinal schedules and increasing delivery costs. Another drawback of current vaccines, notably those directed against bacterial diseases, is the need for parenteral administration, although most bacterial pathogens infect the host following mucosal colonization and invasion.

Recent advances in vaccine technology may help to overcome these drawbacks. For instance, encapsulation of vaccines in biodegradable microspheres composed of poly(DL-lactide-co-glycolide) (DL-PLG) results in stable and effective mucosal immunogens (8, 35). Recently, it was shown that intranasal immunization of mice with low doses of encapsulated pertussis antigens prior to *Bordetella pertussis* infection reduced bacterial recovery by 3 $\log_{10}$ CFU in lung homogenates (35). This result underlines the potential of DL-PLG microsphere vaccines against bacteria which infect the respiratory mucosa of the host.

Others showed that when pilus proteins of rabbit diarrheogenic *Escherichia coli* were incorporated into biodegradable DL-PLG microspheres and administered intraduodenally, bacterial attachment to cecal epithelium in a rabbit diarrhea model dropped significantly (27). However, whether DL-PLG microspheres can be used to elicit protection against infections caused by bacteria which actually invade the host through mucosa such as that of the intestine has not, as far as we know, been investigated.

Unlike the rabbit diarrhea model, oral experimental infection of mice by *Salmonella typhimurium* is a well-established model of intestinal invasive disease (29). This is of interest because we previously showed that after oral immunization with low doses of live *S. typhimurium* (30), a specific immune response to phosphorylcholine (PC), a ubiquitous antigen present on different pathogenic bacteria such as *Streptococcus pneumoniae* (2), *S. typhimurium* (30), *Proteus morganii* (11), and *Neisseria meningitidis* (17), could be induced in mice in both serum and intestinal secretions. Moreover, in mice infected by *Streptococcus pneumoniae*, anti-PC immunity proved protective against infection caused by this PC-bearing bacteria (6, 7, 36, 37).

Therefore, PC, which already appears promising in the development of a vaccine which could be a candidate for pneu-

* Corresponding author. Mailing address: Laboratoire de Bactériologie, Groupe Hospitalier Bichat-Claude Bernard, 46 Rue Henri-Huchard, 75877 Paris Cedex 18, France. Phone: 33 1 40 25 85 00. Fax: 33 1 42 28 99 51. E-mail: andremont-a@bch.ap-hop-paris.fr.

† Present address: Centre de Recherche Nestle, Lausanne 1000, Switzerland.

mococcal prophylaxis (20), might also constitute a model antigen for addressing the issue of useful cross-protection against unrelated invasive diseases caused by other PC-bearing bacteria.

In the present study, we used an oral vaccine comprising PC coupled to porcine thyroglobulin and entrapped in DL-PLG microspheres, and we showed that the anti-PC immunity it elicited in BALB/c mice promoted acquired immunity against a lethal oral challenge by *S. typhimurium* C5.

MATERIALS AND METHODS

Antigen preparation. Paradiazonium phenyl phosphorylcholine (DPPC) was prepared at 4°C from *p*-aminophenyl phosphorylcholine (Sigma Chemical Co., St. Louis, Mo.) and sodium nitrite as described previously (9). DPPC was then conjugated, as described earlier (10), either to porcine thyroglobulin (Sigma; PC-thyr) for use in immunization procedures after entrapment in microspheres or to fraction V bovine serum albumin (Boehringer GmbH, Mannheim, Germany; PC-BSA) to be used for an enzyme-linked immunosorbent assay (ELISA). The PC-to-protein molar ratios of the conjugates were estimated by a microphosphate assay as described previously (1) to be 6 and 108 for PC-BSA and PC-thyr, respectively (data not shown).

Microsphere preparation. Microspheres of DL-PLG (ratio, 75:25; molecular size, 128,000 Da; Birmingham Polymers, Birmingham, Ala.) were prepared by multiple-solvent evaporation emulsion as described previously (4). They were loaded as described before (4) with PC-thyr at a PC-thyr–to-polymer ratio of 1:8 (wt/wt). Blank microspheres were prepared identically in the absence of PC-thyr. Microspheres were collected by 5 min of centrifugation at 700 × *g*, washed three times with distilled water, and freeze-dried in 0.1% polyvinyl alcohol. Diameters, measured as described previously (4), were ≤10 μm for 94 and 97% of the microspheres that were loaded with PC-thyr or unloaded, respectively. The amount of encapsulated thyroglobulin was assayed after release of the conjugate from 30 mg of microspheres incubated in 3 ml of 0.1 N NaOH overnight with stirring (21). The encapsulation efficiency was 88% (data not shown). The 475-nm specific absorbance spectra of the PC-thyr conjugate were identical before and after encapsulation, as were its PC-specific ELISA reactivities (data not shown; see below for methods).

Mice. Female 8-week-old BALB/c mice (IFFA-CREDO, Saint-Germain sur l'Arbresle, France) were kept in groups of six in filter-topped cages with sterile litter, food, and water in a biosafety animal facility.

Immunization procedures. Mice were randomly assigned to one of four immunization groups (six mice per group). The first two groups were immunized on 3 consecutive days by intragastric administration of 500 μl of saline containing 75 mg of DL-PLG microspheres either loaded with PC-thyr (280 μg of PC) or unloaded. A third group of mice received free PC-thyr suspended in 500 μl of saline under similar conditions. Three identical booster doses were administered intragastrically 29, 30, and 31 days later as described previously (8). The fourth group of mice was immunized intraperitoneally with PC-thyr (15 μg of PC) diluted 0.8:10 in complete Freund's adjuvant (Sigma) and was boosted intraperitoneally at days 10, 14, and 20 with PC-thyr without adjuvant as described previously (30). Nonimmunized control mice were maintained under the same conditions.

Evaluation of the immune response. The anti-PC immunoglobulin G (IgG), IgA, and IgM responses were assayed 14 days after the last boost in mice immunized intragastrically and 25 days after the last boost in those immunized intraperitoneally, i.e., in all instances, 45 days after the first immunization dose.

After intragastric immunization with PC-thyr-loaded microspheres, the kinetics of the IgA immune response were measured further in a separate experiment in which 18 mice were immunized as described above and killed by cervical dislocation in three groups of 6 mice on days 5, 15, and 25 after the last boost. Blood samples were collected by retro-orbital puncture. Sera were diluted 1:40 in bicarbonate buffer. Intestinal contents were collected immediately after death as described previously (16). We considered that the intestinal fluid sample dilution was 1:5 (16). Blood and intestinal samples were rapidly frozen at −80°C until used.

Antibody assays. Twofold dilutions of serum and intestinal samples were assayed in duplicate for anti-PC IgM, IgG, and IgA by ELISA, as described previously (10, 30), with PC-BSA-coated plates and goat anti-mouse IgA, IgG, or IgM conjugated to alkaline phosphatase (Sigma). Titers of test samples were determined by calculating the dilution of the sample which gave an absorbance at 405 nm equivalent to that of negative controls consisting either of pooled sera or of pooled intestinal samples from 40 nonimmunized mice and which were included in each assay. Titers were expressed as the $\log_{10}$ of the reciprocal of the dilution and compared between groups by Student's *t* test.

Avidity index. The avidities of anti-PC IgA in serum and intestinal secretions for PC-BSA were compared, as described previously (32), with slight modifications (5). Results were expressed as percentages of the optical density values at 405 nm obtained with control wells incubated with 100 μl of 2 M thiocyanate before incubation with the samples.

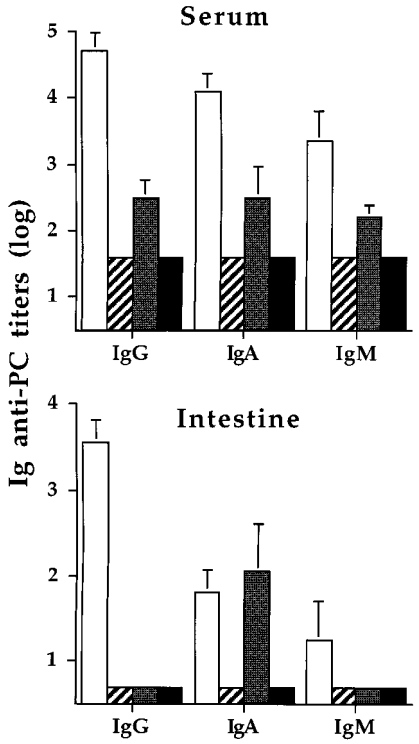

FIG. 1. Anti-PC IgG, IgA, and IgM antibody titers determined in serum and intestinal secretions 45 days after primer immunization of mice receiving PC-thyr intraperitoneally (□) or free PC-thyr (▨), PC-thyr-loaded microspheres (▤), or blank microspheres (■) intragastrically. Each value is the mean ± standard error for six mice. Ig, immunoglobulin.

Oral challenge. Oral infection with *S. typhimurium* C5 (kindly provided by M. Y. Popoff, Institut Pasteur, Paris, France) was performed, as described previously (29), at the peak of the IgA anti-PC response. Briefly, mice were deprived of water overnight and then given free access for 18 h to a drinking bottle containing 100 ml of the inoculum. Afterwards, the inoculum was replaced by acidic sterile water (pH 3). Although this technique did not guarantee that the inocula were strictly identical in all mice, it was chosen because it eliminated all possibility of esophageal injury and subsequent bacterial dissemination, which are liable to occur when the inoculum is delivered via gastric tubing. Preliminary experiments showed that an inoculum of 10^5 bacteria/ml induced 80 to 100% mortality rates within 12 to 15 days postchallenge (data not shown). This inoculum density was used in all further experiments. Bacterial counts in the drinking bottles did not vary significantly between the beginning and end of the inoculation period (data not shown).

The number of animals that died was recorded daily for 4 weeks postchallenge. To eliminate observer bias, the mice which had undergone the various immunization procedures were placed in cages marked only with a code letter. The code was unknown to all experimenters, including those recording the deaths. It was broken 4 weeks after challenge, and the between-group differences in death rates were then compared by the log-rank test.

RESULTS

Serum and intestinal anti-PC immune response. The anti-PC IgG, IgA, and IgM titers measured after the various immunization procedures (Fig. 1) show that the IgA increased in serum and intestinal secretions both after intraperitoneal injection of PC-thyr and after intragastric administration of PC-thyr-loaded microspheres. The mean serum IgA titers observed following intragastric immunization with PC-thyr-loaded microspheres were significantly lower ($P < 0.001$) than the titers observed after intraperitoneal immunization with PC-thyr. However, the IgA titers in the intestinal secretions were similar in the two groups of mice, whatever the route and formulation of the antigen. However, the ratio between intes-

TABLE 1. Ratio of immunoglobulin titers obtained in serum and in intestinal secretions of mice immunized intraperitoneally (i.p.) with PC-thyr and intragastrically (i.g.) with PC-thyr-loaded microspheres

Immunoglobulin	Titer ratio[a]	
	i.p.	i.g.
IgA	0.44	2.05
IgG	0.74	0.27
IgM	0.37	0.31

[a] Intestinal secretion/serum immunoglobulin titer ratio.

tinal secretions and serum IgA titers was higher for mice immunized intragastrically with PC-loaded microspheres than for mice immunized intraperitoneally, in contrast to the ratio of IgG titers (Table 1). The functional avidities of anti-PC IgA, as measured by thiocyanate dissociation, were not significantly different between mice immunized intraperitoneally with PC-thyr and those immunized orally with PC-thyr-loaded microspheres (Fig. 2).

By contrast, intestinal and systemic IgG and IgM responses were much lower after intragastric than intraperitoneal immunization. No increase in IgG, IgA, and IgM was observed in serum or intestinal secretions after intragastric administration of free PC-thyr. All serum and intestinal samples from the mice given blank microspheres were negative.

Kinetics of anti-PC IgA titers. The anti-PC IgA titers assayed at various time intervals after intragastric immunization with PC-thyr-loaded microspheres exhibited a peak (Fig. 3) 2 weeks after administration of the last intragastric immunization dose, both in serum and in intestinal secretions. The amplitude of this peak was greater in the intestinal secretions. The day at which anti-PC IgA titers peaked was therefore chosen as the time of challenge in the protection experiments.

Protection after oral challenge by *S. typhimurium* C5. Death rates were recorded for the various immunization groups after oral challenge by *S. typhimurium* C5. The results (Fig. 4) show that the mice immunized orally with PC-thyr-loaded microspheres were significantly better protected against this challenge than those which were intragastrically immunized with unloaded microspheres ($P < 10^{-4}$), free PC-thyr ($P = 0.0003$), or parenteral PC-thyr ($P = 0.003$).

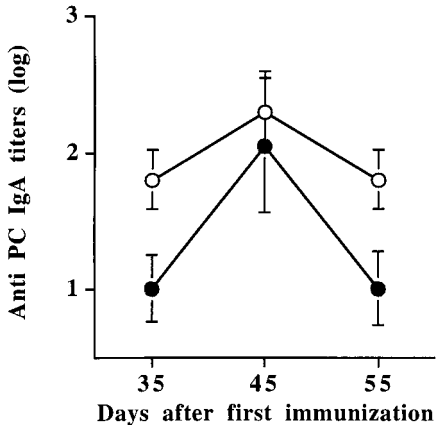

FIG. 3. Kinetics of anti-PC IgA antibody titers in serum (○) and intestinal secretions (●) of mice immunized intragastrically with PC-thyr-loaded microspheres (see Materials and Methods for details). Each value is the mean ± standard error for six mice.

The death rate in mice immunized intraperitoneally with PC-thyr was significantly lower than that observed in those given blank microspheres ($P = 0.009$), but the difference between the death rates in the group given blank microspheres and the group immunized intragastrically with free PC-thyr was not significant ($P = 0.18$).

DISCUSSION

Our results showed that PC-thyr-loaded microspheres are potent immunogens promoting both local and systemic anti-PC immune responses. In addition, a highly significant degree of acquired immunity against an oral challenge with a lethal dose of *S. typhimurium* was obtained in BALB/c mice after intragastric immunization with PC-thyr-loaded microspheres. We achieved this immunity by use of a previously recommended oral immunization procedure that included neither parenteral priming nor boosting (8). The rationale for this option was that this immunization procedure had the greatest potential in terms of vaccine development, although the combination of both the oral and parenteral routes has been shown to be synergistic (24).

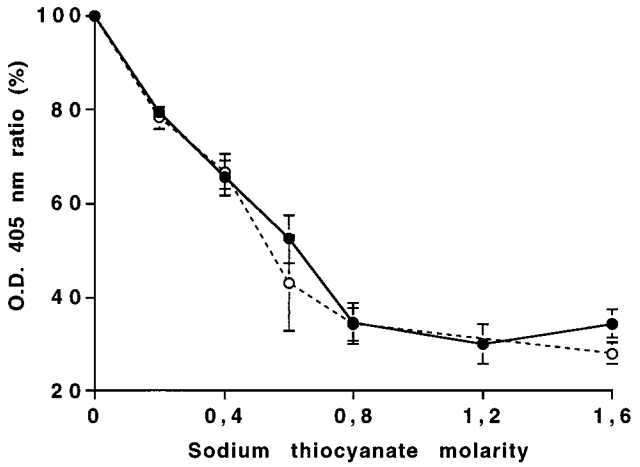

FIG. 2. Comparative avidity curves of IgA anti-PC in intestinal secretions of mice immunized intraperitoneally with PC-thyr (○) or PC-thyr-loaded microspheres (●). OD, optical density.

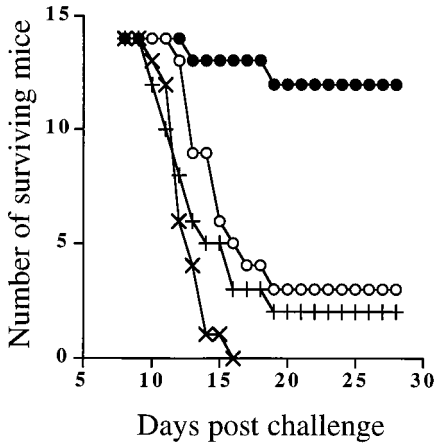

FIG. 4. Cumulative mortality rates after oral challenge by *S. typhimurium* C5 of mice immunized intraperitoneally with PC-thyr (○) or intragastrically with free PC-thyr (+), PC-thyr-loaded microspheres (●), or blank microspheres (×).

Mucosal immunization without parenteral injection has also been used by others to protect mice against mucosal colonization by *Helicobacter pylori* (18), *B. pertussis* (35), or *E. coli* (27). In the last two of these models, in which the antigens were encapsulated in DL-PLG microspheres, the efficacy of the vaccine appeared to be due to the increase in IgA levels, in respiratory secretions (35), or in the bile (27). In the particular model tested in the present study, we also observed both protection against *S. typhimurium* infection and elevated serum and intestinal anti-PC IgA after intragastric administration of PC-thyr-loaded microspheres.

It was not surprising to observe that the IgG and IgM responses after intragastric immunization with PC-thyr-loaded microspheres were weak because mucosal immunization is known to induce, essentially, a rise in IgA titers (3, 30, 34).

The combined increases in both the IgA response and the protective immunity observed after intragastric immunization with PC-thyr-loaded microspheres were strikingly larger than those observed after oral immunization with free PC-thyr. This suggests that DL-PLG microspheres preserved the integrity of PC adequately during gastrointestinal transit and allowed appropriate uptake by the intestinal immune system.

Since no protection was observed after oral administration of blank microspheres, protective immunity observed after intragastric immunization with PC-thyr-loaded microspheres appeared to be due to the anti-PC immune response.

We also observed that the degree of acquired protection achieved after intraperitoneal immunization with PC-thyr was significantly lower than that acquired after intragastric immunization with PC-thyr-loaded microspheres. This might be because a smaller amount of antigen was administered intraperitoneally than intragastrically or because the infectious challenge was done at a later date after the last boost in the mice immunized intraperitoneally than in those immunized intragastrically. However, at the time of challenge, significantly higher anti-PC IgG and IgM titers were present in both the sera and intestinal secretions from the intraperitoneally immunized mice. In addition, intestinal anti-PC IgA titers were similar in both groups. Moreover, the avidities of these anti-PC antibodies, as measured by a thiocyanate dissociation assay, were not significantly different. Thus, possible explanations for the lower degree of protection observed after intraperitoneal immunization might be either that other immune mechanisms are important for protection or that the IgA being measured is somehow not functional for protection.

A large fraction of the anti-PC IgA present in the intestines of the mice immunized intraperitoneally may indeed be of extraintestinal origin. Thus, in rodents, transport by the liver of serum IgA into the intestine is an important source of intestinal IgA (19). This occurs via the expression, on the liver sinusoidal surface, of the receptor of polymeric immunoglobulin, the parent protein of the secretory component. These bile-excreted secretory IgA (sIgA) might be less effective in preventing invasion by *S. typhimurium* than the mucosal sIgA present in the intragastrically immunized mice because, at present, mucosal sIgA is believed to function at three levels to protect the host (26). In addition to protecting the host within the intestinal lumen, mucosal sIgA also protects the host inside the epithelial cells (28) lining the mucous membrane, as demonstrated with viruses (25, 26), and in the lamina propria, in which numerous plasma cells secrete dimeric sIgA (22, 23). Obviously, the sIgA excreted by the bile would not function inside epithelial cells or in the lamina propria, which might explain their reduced protective efficacy. The higher ratio of intestinal secretion/serum IgA titers in intragastrically immunized mice than in those immunized intraperitoneally strongly suggests that some of the IgA in the former group has a secretory origin.

The precise location of PC in *S. typhimurium* cells is not elucidated. However, in *Morganella (Proteus) morganii*, another member of the *Enterobacteriaceae* family, PC is carried by an immunodominant oligosaccharide (31) which has been reported to be an integral part of the bacterial lipopolysaccharide (38).

The role of DL-PLG microspheres in enhancing mucosal antibody responses and acquired resistance is not fully understood. The physical conformation of the polymer-bound antigen may redirect immune responses by influencing antigen recognition and processing by lymphoid or myeloid antigen-presenting cells. Also, antigen uptake and release by M cells change upon encapsulation in microspheres, and the intensity of the mucosal response appears to be dependent on microsphere diameter (12–14). The diameters of the microspheres used in the present work were less than 10 μm, i.e., equivalent to those of the microspheres used by others to protect rabbits against *E. coli* intestinal colonization (27, 33).

It is of interest that the DL-PLG microspheres used intranasally to protect mice against lung infection by *B. pertussis* were also similar in diameter (35) because PC, the model antigen used in our study, is known to elicit protection against another PC-bearing bacteria, namely *Streptococcus pneumoniae* (6, 7, 20, 36, 37), which also primarily infects the lungs during the course of natural disease. Thus, one can hypothesize whether a similar anti-PC vaccine, particularly if administered intranasally, would be protective against *Streptococcus pneumoniae.*

Overall, our finding that PC encapsulated in DL-PLG microspheres is an effective mucosal vaccine against murine intestinal infection with *S. typhimurium* suggests that the efficacy of a similar strategy should be investigated in models of diseases caused by other PC-bearing microorganisms.

ACKNOWLEDGMENTS

We thank J. M. Alonso and P. Ernst for expert revision of the manuscript, J. P. Bouvet for discussions, M. Y. Popoff for the gift of the bacterial strain used for challenge experiments, and Florence Chrétien and Evelyne Dumarquez for technical assistance.

REFERENCES

1. **Bartlett, G. R.** 1959. Phosphorus assay in column chromatography. J. Biol. Chem. **243:**466–468.
2. **Beckmann, E., and D. Lewitt.** 1984. Phosphorylcholine on *Streptococcus pneumoniae* R36a is responsible for *in vitro* polyclonal antibody secretion by human peripheral blood lymphocytes. J. Immunol. **132:**2174–2176.
3. **Bergmann, K. C., and R. H. Waldman.** 1988. Stimulation of secretory antibody following oral administration of antigen. Rev. Infect. Dis. **10:**939–950.
4. **Blanco-Prieto, P. M., F. Delie, E. Fattal, A. Tartar, F. Puisieux, A. Gulik, and P. Couvreur.** 1994. Characterization of V3 BRU peptide-loaded small PLGA microspheres prepared by a (W_1/O/W_2) emulsion solvent evaporation method. Int. J. Pharm. **111:**137–145.
5. **Bouvet, J. P., L. Bélec, R. Pirès, and J. Pillot.** 1994. Immunoglobulin G antibodies in human vaginal secretions after parenteral vaccination. Infect. Immun. **62:**3957–3961.
6. **Briles, D. E., C. Forman, S. Hudak, and J. L. Claflin.** 1984. The effects of subclass on the ability of anti-phosphorylcholine antibodies to protect mice from fatal infections with *Streptococcus pneumoniae*. J. Mol. Cell. Immunol. **1:**305–309.
7. **Briles, D. E., J. Horowitz, L. S. McDaniel, W. H. J. Benjamin, L. Claflin, C. Booker, G. Scott, and C. Forman.** 1986. Genetic control of the susceptibility to pneumococcal infection. Curr. Top. Microbiol. Immunol. **124:**103–120.
8. **Challacombe, S. J., D. Rahman, H. Jeffery, S. S. Davis, and D. T. O'Hagan.** 1992. Enhanced secretory IgA and systemic IgG antibody responses after oral immunization with biodegradable microparticles containing antigen. Immunology **76:**164–168.
9. **Chesebro, B., and H. Metzger.** 1972. Affinity labeling of a phosphorylcholine-binding mouse myeloma protein. Biochemistry **11:**766–771.
10. **Claflin, J., R. Lieberman, and J. Davie.** 1974. Clonal nature of the immune

response to phosphorylcholine. I. Specificity, class, and idiotype of phosphorylcholine-binding receptors on lymphoid cells. J. Exp. Med. **134:**188–200.
11. **Claflin, J. L., J. George, C. Dell, and J. Berry.** 1989. Patterns of mutations and selection in antibodies to the phosphocholine-specific determinant in *Proteus morganii*. J. Immunol. **143:**3054–3063.
12. **Eldridge, J. H., R. M. Gilley, J. K. Staas, Z. Moldoveanu, J. A. Meulbroek, and T. R. Tice.** 1989. Biodegradable microspheres: vaccine delivery system for oral immunization. Curr. Top. Microbiol. Immunol. **14:**59–66.
13. **Eldridge, J. H., J. A. Meulbroek, J. K. Staas, T. R. Tice, and R. M. Gilley.** 1989. Vaccine-containing biodegradable microspheres specifically enter the gut-associated lymphoid tissue following oral administration and induce a disseminated mucosal immune response. Adv. Exp. Med. Biol. **251:**192–202.
14. **Eldridge, J. H., J. K. Staas, J. A. Meulbroek, J. R. McGhee, T. R. Tice, and R. M. Gilley.** 1991. Biodegradable microspheres as a vaccine delivery system. Mol. Immunol. **28:**287–294.
15. **Eldridge, J. H., J. K. Staas, J. A. Meulbroeck, T. R. Tice, and R. M. Gilley.** 1991. Biodegradable and biocompatible poly (DL-lactide-co-glycolide) microspheres as an adjuvant for staphylococcal enterotoxin B which enhances the levels of toxin-neutralizing antibodies. Infect. Immun. **59:**2978–2986.
16. **Elson, C., W. Ealding, and J. Lefkowitz.** 1984. A lavage technique allowing repeated measurement of IgA antibody in mouse intestinal secretions. J. Immunol. Methods **67:**101–108.
17. **Faro, J., R. Seoane, A. Eiras, I. Lareo, J. Couceiro, and B. J. Regueiro.** 1986. Immunoresponses to *Neisseiria meningitidis* epitopes: suppression of secondary response to phosphorylcholine is carrier specific. Infect. Immun. **51:**224–232.
18. **Ferrero, R. L., J. M. Thiberge, I. Kansau, M. Wuscher, M. Huerre, and A. Labigne.** 1995. The GroES homolog of *Helicobacter pylori* confers protective immunity against mucosal infection in mice. Proc. Natl. Acad. Sci. USA **92:**6499–6503.
19. **Fisher, M. M., B. Nagy, H. Bazin, and B. J. Underdown.** 1979. Biliary transport of IgA: role of secretory component. Proc. Natl. Acad. Sci. USA **76:**2008–2012.
20. **Fisher, R. T., D. L. Longo, and J. J. Kenny.** 1995. A novel phosphocholine antigen protects both normal and X-linked immune deficient mice against *Streptococcus pneumoniae*. J. Immunol. **154:**3373–3382.
21. **Jeffery, H., S. S. Davis, and D. T. O'Hagan.** 1993. The preparation and characterization of poly(lactide-co-glycolide) microparticles. II. The entrapment of a model protein using a (water-in-oil)-in-water emulsion solvent evaporation technique. Pharm. Res. **10:**362–368.
22. **Kaetzel, C. S., J. K. Robinson, K. R. Chintalacharuvu, J. P. Vaerman, and M. E. Lamm.** 1991. The polymeric immunoglobulin receptor (secretory component) mediates transport of immune complexes across epithelial cells: a local defense function for IgA. Proc. Natl. Acad. Sci. USA **88:**8796–8800.
23. **Kaetzel, C. S., J. K. Robinson, and M. E. Lamm.** 1994. Epithelial transcytosis of monomeric IgA and IgG cross-linked through antigen to polymeric IgA. A role for monomeric antibodies in the mucosal immune system. J. Immunol. **152:**72–76.
24. **Keren, D. F., R. A. McDonald, and J. L. Cary.** 1988. Combined parenteral and oral immunization results in an enhanced mucosal immunoglobulin A response to *Shigella flexneri*. Infect. Immun. **56:**910–915.
25. **Mazanec, M. B., C. S. Kaetgel, M. E. Lamm, D. Fletcher, and J. G. Nedrud.** 1992. Intracellular neutralization of virus by immunoglobulin A antibodies. Proc. Natl. Acad. Sci. USA **89:**6901–6905.
26. **Mazanec, M. B., J. G. Nedrud, C. S. Kaetzel, and M. E. Lamm.** 1993. A three-tiered view of the role of IgA in mucosal defense. Immunol. Today **14:**430–435.
27. **McQueen, C. E., E. C. Boedeker, R. Reid, D. Jarboe, M. Wolf, M. Le, and W. R. Brown.** 1993. Pili in microspheres protect rabbits from diarrhoea induced by *E. coli* strain RDEC-1. Vaccine **11:**201–206.
28. **Mostov, K. E., and D. L. Deitcher.** 1986. Polymeric immunoglobulin receptor expressed in MDCK cell transcytoses IgA. Cell **46:**613–621.
29. **Pardon, P., M. Y. Popoff, C. Coynault, J. Marly, and I. Miras.** 1986. Virulence-associated plasmids of *Salmonella* serotype typhimurium in experimental murine infection. Ann. Inst. Pasteur/Microbiol. **137B:**47–60.
30. **Pecquet, S. S., C. Ehrat, and P. Ernst.** 1992. Enhancement of mucosal antibody responses to *Salmonella typhimurium* and the microbial hapten phosphorylcholine in mice with X-linked immunodeficiency by B-cell precursors from the peritoneal cavity. Infect. Immun. **60:**503–509.
31. **Potter, M.** 1971. Antigen binding myeloma proteins in mice. Ann. N. Y. Acad. Sci. **190:**306–321.
32. **Pullen, G. R., M. G. Fitzgerald, and C. S. Hosking.** 1986. Antibody avidity determination by ELISA using thiocyanate elution. J. Immunol. Methods **86:**83–87.
33. **Reid, R. H., E. C. Boedeker, C. E. McQueen, D. Davis, L. Y. Tseng, J. Kodak, K. San, C. L. Wilhelmsen, R. Mellore, P. Dalal, and H. R. Bhagat.** 1993. Preclinical evaluation of microencapsulated CFA/II oral vaccine against enterotoxigenic *E. coli*. Vaccine **11:**159–167.
34. **Scott, R. M., B. A. Dudding, S. V. Romano, and P. K. Russel.** 1972. Enteric immunization with live adenovirus type 21 vaccine. II. Systemic and local immune responses following immunization. Infect. Immun. **5:**300–304.
35. **Shahin, R., M. Leef, J. Eldridge, M. Hudson, and R. Gilley.** 1995. Adjuvanticity and protective immunity elicited by *Bordetella pertussis* antigens encapsulated in poly(DL-lactide-co-glycolide) microspheres. Infect. Immun. **63:**1195–1200.
36. **Wallick, S., J. L. Claflin, and D. E. Briles.** 1983. Resistance to *Streptococcus pneumoniae* is induced by a phosphocholine-protein conjugate. J. Immunol. **130:**2871–2875.
37. **Yother, J., C. Forman, B. M. Gray, and D. E. Briles.** 1982. Protection of mice from infection with *Streptococcus pneumoniae* by anti-phosphocholine antibody. Infect. Immun. **36:**184–188.
38. **Young, M. N., and J. C. Richard.** 1994. Structural characterisation of the phospohocholine-bearing antigen from *M. morganii* and its recognition by monoclonal antibodies, p. 66. *In* Abstracts of the XVIIth International Carbohydrate Symposium, Ottawa, Canada.

Editor: J. R. McGhee