specific IgG2a, and IFN-γ secretion by the Th cells in the β-gal/alum-primed mice. However, despite the recent success of DNA-based immunization in altering the IgE- and Th2-associated immune response in various models, the prophylactic and/or therapeutic potentials are far from clear, and better, more efficient, and safer gene transfer and gene expression system by using improved DNA delivery systems are urgently needed.

Gene therapy continues to hold exciting promise in treating many genetic disorders [36,37]. Recent clinical trials indicate that an efficient and safe delivery vehicle remains a crucial barrier to successful gene therapy [38]. Viral and retroviral vectors have been the most efficient and commonly used delivery modalities for in vivo gene transfer [39–41]. However, issues of immune response to viral proteins remain to be addressed. Non-viral delivery systems have been increasingly proposed as alternatives to viral vectors because of potential advantages such as ease of synthesis, cell/tissue targeting, low immune response, and unrestricted plasmid size.

The most promising non-viral gene delivery system thus far, other than the "gene gun" in DNA vaccine applications, comprises ionic complexes formed between DNA and polycationic liposomes [42–45]. Factors hindering the success of the liposomal approach appear to be instability of the complex, toxicity of the cationic lipid, and short half-life of the complexed DNA. Held together by electrostatic interaction, these complexes may dissociate because of the charge screening effect of the polyelectrolytes in the biological fluid. A strongly basic lipid composition can stabilize the complex, but such lipids may be cytotoxic. The fact that the DNA is coated on the outside of the liposome renders the DNA vulnerable to nuclease degradation in transit from site of administration. to the nucleus of the target cell. A recent pharmacokinetic study shows that a HLA-B7 gene, when delivered by DC-Cho1/DOPE intravenously, has a half-life of less than five minutes, and becomes undetectable after one hour [46]. No encoded protein could be detected in tissues harboring the residual plasmid by immunohistochemical analysis at 1 or 7 days post-injection. This poor bioavailability is not too surprising in light of similar instability which is characteristic of many therapeutic proteins. For example, interferons and several other cytokines also have plasma half-lives in the order of minutes. The therapeutic potential of many of these delicate polypeptides has been improved by controlled release formulations.

Complex coacervation is a process of spontaneous phase separation that occurs when two oppositely charged polyelectrolytes are mixed in an aqueous solution. The electrostatic interaction between the two species of macromolecules results in the separation of a coacervate (polymer-rich phase) from the supernatant (polymer-poor phase). This phenomenon can be used to form microspheres and encapsulate a variety of compounds. The encapsulation process can be performed entirely in aqueous solution and at low temperatures, and has a good chance, therefore, of preserving the bioactivity of the encapsulant. In developing an injectable controlled release system, we have used the complex coacervation of gelatin and chondroitin sulfate to encapsulate a number of drugs and proteins [47]. Cytokines have been encapsulated in these microspheres for cancer vaccination [48]. Anti-inflammatory drugs have also been incorporated for intra-articular delivery to the joints for treating osteoarthritis [49]. It occurred to us that we could replace chondroitin sulfate with DNA as the polyanion to form microspheres with gelatin or other polycations.

Gelatin, the denatured form of collagen, is a polyampholyte which gels below 35–40° C. It is widely used in food (55%), pharmaceutical (25%), and photographic (15%) industries [50]. At pH below 5, it is positively charged and can complex with DNA to form a coacervate. After crosslinking, the coacervate is stable in solution of high ionic strength. Chitosan is a bioabsorbable, non-toxic, polysaccharide with low. immunogenicity. The polysaccharide has an average amino group density of 0.837 per disaccharide unit. It has been proposed as a matrix for controlled release microsphere formulation and as a stabilizer for alginate capsules encapsulating cells.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a vaccine preparation suitable for oral administration.

It is another object of the present invention to provide a method of immunizing a mammal by ingestion of the vaccine.

These and other objects of the invention are achieved by providing a vaccine formulation which is suitable for oral ingestion. The vaccine comprises a solid nanoparticle of less than 5 μm comprising a coacervate of a polymeric polycation and a polyanion, wherein the polyanion consists of nucleic acids encoding an antigen. A pharaceutically acceptable vehicle may be used as a carrier.

According to another embodiment a method is provided of vaccinating a mammal against an antigen. The method comprises: orally administering a vaccine formulation comprising a solid nanoparticle of less than 5 μm. The nanoparticle comprises a coacervate of a polymeric polycation and a polyanion. The polyanion consists of nucleic acids encoding an antigen.

These and other embodiments provide the art with new tools for simply and effectively vaccinating people and other mammals against inter alia viral, parasitic, fungal, and bacterial diseases. Environmental and food allergens can also be used as the antigens.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**. Immune response in AKR/J mice orally immunized with various formulations of DNA-chitosan nanospheres and naked DNA encoding the Peanut allergen. Top panel: IgG response in serum 4 and 6 weeks after immunization. Purified mouse IgG was used as standard to calculate titer (ng). Bottom Panel: IgA response in fecal extract 3 and 4 weeks after immunization. O.D valuse at 490 nm is reported here for undiluted sample (supernatant of 100 mg/ml fecal pellets dissolved in PBS). Each bar represents values of pooled samples from eight animals (five in case of negative controls).

FIG. **2**. Protection Studies. Sham-fed and pArah2-nanospheres immunized AKR/J mice were sensitized four times, one week apart, with oral and intra-peritoneal doses of crude peanut extract. Following sensitization the mice were challenged with 1 mg/mice intra-peritoneal dose of pure Arah2 protein and examined for anaphylactic response over a period of time. Right panel: Grade of anaphylaxis of mice over a period of one hour. Left panel: Comparison of different groups 30 minute after challenge. C390=High molecular weight chitosan. PCL113=Low molecular weight chitosan. Each circle represents one mouse.

FIG. **3**. FIG. **3***a*. shows a transmission electron micrograph of chitoson-DNA nanoparticles. Freshly prepared particles were adsorbed on a glow discharged carbon grid, air-dried and rotary shadowed with platinum at 7°. The grid was viewed under a Zeiss transmission electron microscope