Polyanhydride nanovaccine elicits germinal center formation and isotype switching

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Statement of Purpose: In the development of prophylactic tools to prevent disease, the design of vaccines with novel adjuvant formulations that can elicit strong and balanced immune responses towards pathogens is an important goal. Biodegradable polymeric nanoparticles have shown promising characteristics as adjuvants and/or delivery vehicles by enhancing the immune response compared to soluble protein.[1] Specifically, polyanhydride nanoparticles have successfully encapsulated and released antigens, activated T cells, and elicited antibodies towards a variety of immunogens.[2,3] One of the characteristics in the induction of strong antibody responses is the generation of B cell germinal centers, which is part of the T helper-cell driven cellular response. The goals of this study were to understand the role of these biomaterials in the induction of antigen-specific immune responses, their ability to elicit germinal B cell center formation, isotype switching, and antibody responses after subcutaneous administration.

Methods: Copolymers based on 1,6-bis(*p*-carboxyphenoxy)hexane (CPH) and 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) were synthesized using melt polycondensation, as described previously.[4,5] The antigen of interest in the current studies was gp41-54Q-GHC, an HIV polypeptide, which was encapsulated into polyanhydride nanoparticles via nanoprecipitation with 1% v/v Span80®.[6] BALB/c mice were immunized in the footpad once with 500 µg of 2% gp41-loaded 20:80 CPTEG:CPH nanoparticles. Serum samples were collected at days 0, 8, 12 and 18 along with the draining lymph nodes. Germinal center B cells and T follicular helper cellular populations were quantified in the draining lymph nodes using flow cytometry.[7] In addition, serum antibody levels were measured using an ELISA at the same time points. The responses induced by the nanovaccines were compared with the responses induced by a traditional adjuvant (Alum).

Results: Polyanhydride nanoparticles loaded with 2% gp41-54Q-GHC were successfully synthesized. Draining lymph nodes from mice immunized with polyanhydride nanovaccines or Alum-based formulations showed enhanced generation of germinal B cells and T follicular helper cells, with the response being the highest at day 12 (Fig 1).

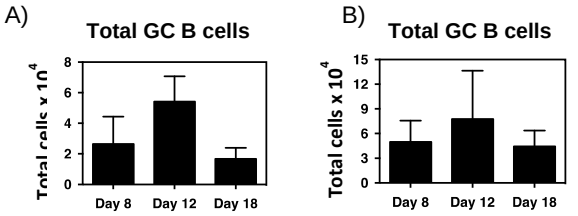

Figure 1. Generation of B cell germinal centers by polyanhydride nanovaccines and Alum in draining lymph nodes. Panel A shows the number of B cells in the draining lymph nodes of BALB/c mice after a single administration of 500 µg of 2% gp41-loaded polyanhydride nanoparticles. Panel B shows the number of B cells in the draining lymph nodes of BALB/c mice after a single administration of the same amount of gp41-antigen as in A adsorbed to Alum.

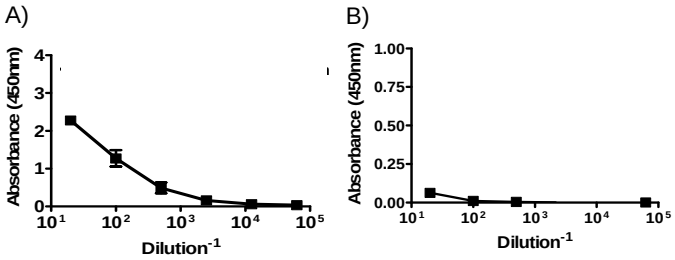

Figure 2. Panel A shows antibody levels in mouse serum quantified using ELISA at day 18 after a single immunization with 500 µg of 2% gp41-loaded polyanhydride nanoparticles. Panel B shows antibody levels in mouse serum quantified using ELISA at day 18 after a single immunization with gp41-antigen adsorbed to Alum.

Single dose immunization with polyanhydride nanovaccines induced a significant production of antibodies compared to Alum at all the time points studied (Fig 2 shows the day 18 data). In addition, polyanhydride formulations induced isotype switching in the generated antibodies. These balanced immune responses have been described not as Th1 nor Th2 phenotypes, but depending upon the secretion of inflammatory cytokines, can be polarized towards the required phenotype.

Conclusions: These studies demonstrate that polyanhydride nanovaccines are capable of eliciting strong germinal B cell responses, inducing isotype switching, and generating robust antibody responses. More significantly, immunization with nanovaccines induced balanced immune responses, in contrast to the response induced by the majority of traditional adjuvants. These studies provide foundational information for the rational design of vaccine formulations against viruses and cancer.

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