Drug Delivery

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Polyacrylate Dendrimer Nanoparticles: A Self-Adjuvanting Vaccine Delivery System**

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Infection with group A streptococci (*Streptococcus pyogenes*, GAS), which is one of the most common and widespread human pathogens, can result in a broad range of diseases, including pharyngitis, with the potential of acute and post-infectious rheumatic fever (ARF) and rheumatic heart disease (RHD).[1] It is estimated that GAS infection is responsible for over half a million deaths per year worldwide.[2] The inability to effectively control GAS infections with antibiotics[3] has prompted extensive research into the development of a vaccine against this infection. However, to date, no prophylactic GAS vaccine is available on the market. Immunity to GAS relies on the production of opsonic antibodies specific to the hypervariable N-terminal and conserved C-terminal regions of the coiled-coil α-helical surface M protein, which is the major virulent factor in GAS.[1,4] The development of an effective vaccine for GAS has been challenged by induced autoimmunity from epitopes derived from the C-terminal regions.[5] The minimal B-cell epitopes are believed to be safe but showed little or no immunogenicity.[6]

It was recently hypothesized that self-assembled amphiphilic polymers can serve as nanoscale delivery systems for subunit vaccines, but no proof of concept has been demonstrated.[7] We report herein a polymer-based vaccine delivery system that offers several potential advantages over previously reported strategies. It was expected that 1) an amphiphilic structure can self-assemble to produce particles with the desired size, 2) the hydrophobic polymer ensures presentation of the peptide epitope on the surface of the nanoparticles, and 3) the dendritic structure and dense packing of epitopes on the surface of the nanoparticles induce native conformation of antigen which is essential for B-cell recognition.

We have synthesized a dendritic structure consisting of a polyacrylate core and a peripheral generation of the minimal B-cell epitope (J14, KQAEDKVKASREAKKQVEKALEQLEDKVK;[8] Figure 1a and Scheme S1 in the Supporting Information). The dendrimer structure, which contains the antigen peptides, resulted in a self-assembled nanoparticle of 20 nm diameter in water. We chose the hydrophobic poly(*tert*-butyl acrylate) as the dendritic core as it had little or no toxicity, was shown to posses adjuvant properties when simply mixed with inactivated viral antigens,[9,10] and has a high affinity for self-assembly into nanoparticles when the peripheral outer layer is hydrophilic.[11] The α-helical 20-mer epitope p145 (LRRDLDASREAKKQVEKALE), which is a peptide from the C-repeat region of the M protein, can elicit a protective antibody immune response when administered with an adjuvant or incorporated into the lipid core of a murine model.[8,12] However, p145 was not a suitable vaccine candidate as T-cells specific to p145 were found to be cross-reactive with human heart tissue,[5] and may prompt the development of autoimmune disease (RHD).[8] Therefore, the J14 epitope used in this work was the minimal B-cell epitope (J14i, ASREAKKQVEKALE; derived from p145) incorporated between two helix-promoting sequences from the yeast GCN4 protein to induce a native conformation. J14i alone had no native secondary helical conformation and produced little or no immunogenicity.[6] The J14 epitope was found to induce protective responses without stimulating cross-reactive antibodies but only when mixed with the toxic complete Freund's adjuvant (CFA).[13]

Previous work using J14 peptides covalently attached to linear polymers (with a broad molecular-weight distribution) generated an antibody response only when coadministered with CFA.[14] Such an imprecise and heterogeneous antigen display combined with CFA makes their mechanistic understanding and regulatory approval challenging. The dendrimer structures described in this work are well-defined (Figure 1). The alkyne-functionalized four-arm star **1** was synthesized by successive atom-transfer radical polymerization (ATRP)[15] and copper-catalyzed alkyne–azide 1,3-dipolar cycloaddition (CuAAC) "click" reaction (Scheme S1 in the Supporting Information).[16] The "living" radical polymerization allowed us to obtain a core with a very narrow molecular-weight distribution (polydispersity index of less than 1.09). Unprotected J14 epitopes possessing an N-terminus azide moiety

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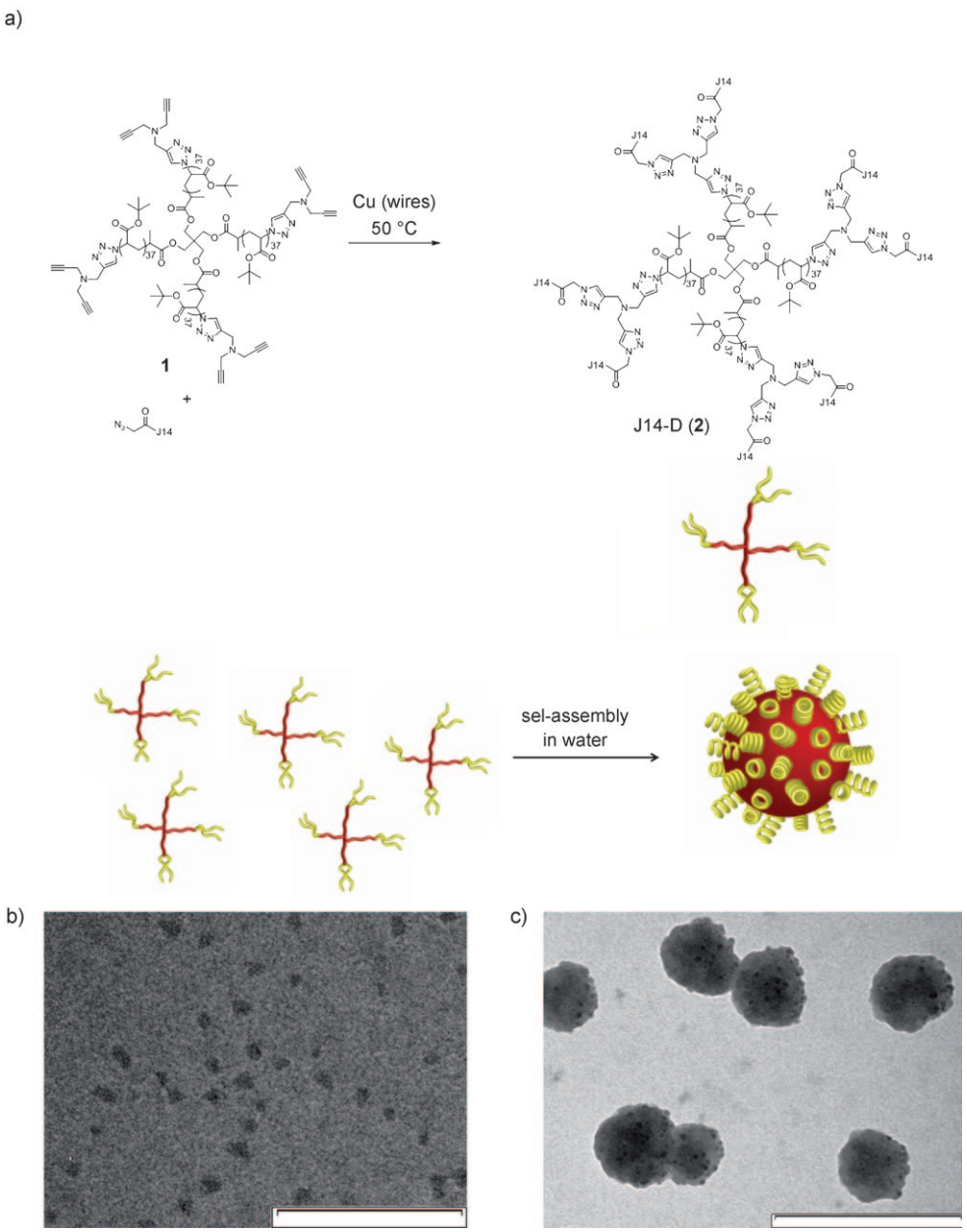

Figure 1. a) Synthesis of dendrimers that contain multiple copies of the J14 epitope (**2**). Yellow ribbons represent the J14 peptide epitope. TEM images of b) **2** and c) immuno-gold-labeled **2** nanoparticles (scale bar: 200 nm).

were further "clicked" onto **1** (Figure 1a and the Supporting Information). We used copper wire instead of the typically copper/ligand catalyst system in order to reduce copper and ligand impurities, and allow ease of purification by simply removing the wire from the reaction medium.[17]

The self-adjuvanting vaccine nanoparticle was formed by dialysis of a solution of the dendrimer in DMF against water. Dialysis also allowed the removal of any remaining peptide, residual copper species from the "click" reaction, and DMF. The attachment of peptide molecules to produce the dendrimer was confirmed by elemental analysis, which showed a significant increase in the nitrogen/carbon ratio to 0.15 compared to that of **1** (N/C = 0.02). These data suggested the conjugation of five peptide epitopes (N/C = 0.15) to a dendritic core. The particle size distribution was narrow, with a particle diameter of 21 nm, based on the volume particle size distribution (> 95 %) of the dendrimer structure (Figure S1 A in the Supporting Information). TEM measurements showed a good correlation with the dynamic light scattering results (Figure 1b). The size of the produced nanoparticles was optimal for vaccine purposes since nanoparticles close to 20 nm in diameter were able to traffic, localize, and maintain a strong presence in lymph nodes, thus allowing the greatest exposure to dendritic cells.[18]

Peptide J14 alone showed a conformation that was more random than helical when measured in water (Figure S2 in the Supporting Information). Under the same conditions, a typical α-helix curve in CD spectra was observed for the dendritic molecule **2**. This result suggests that the conformation of J14 epitopes changed from random to an α-helix on the surface of the nanoparticles (CD spectra of the dendrimer itself were not recorded because of its insolubility in aqueous solvents). The conformation change of the J14 epitope on the nanoparticles was supported by the change in surface charge as measured by the zeta potential, which ranged from 20 mV for the free epitopes to −16 mV for the nanoparticles (Figure S1b in the Supporting Information). To confirm that the J14 epitopes on the surface of the nanoparticles were indeed active and presented in the correct conformation, the nanoparticles were labeled with immunoglobulin G (IgG; which is specific to J14) and reacted with gold-labeled goat anti-mouse antibody. The TEM micrograph (Figure 1c) showed the agglomeration between nanoparticles and gold (black dot) through antibody recognition, thus indicating the presence of active J14 epitopes on the nanoparticle surface.

We measured antibody titers in mice sera following subcutaneous immunization. The nanoparticles produced

high levels of systemic J14-specific IgG antibody similar to that observed with the injection of J14 epitope with CFA as a positive control. Significantly higher antibody titers were observed for the nanoparticles conjugated with J14 epitopes (**2**) in comparison with a physical mixture of J14 with dendrimer **1**, thus suggesting that chemical conjugation of epitopes with a polymer core was essential to elicit an immune response (Figure 2). This observation is in contrast to

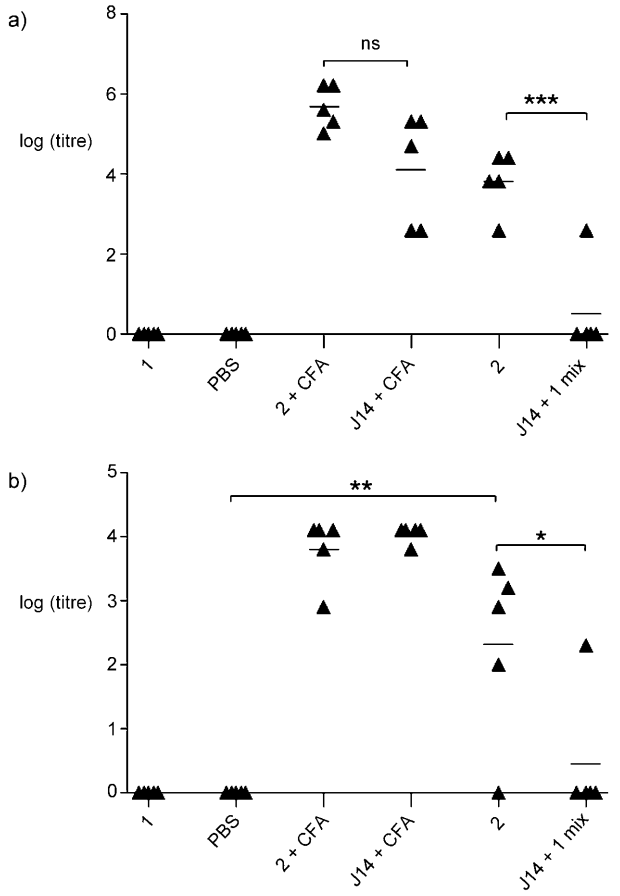

Figure 2. a) J14-specific serum IgG titers and b) p145-specific serum IgG titers at the final bleed (day 37) after subcutaneous immunization for each individual mouse. Mean specific serum IgG antibody titers are represented as a bar. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by the Tukey post hoc test (ns $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

previously reported data, where a physical mixture of antigens with a linear polyacrylate gave significant antibody responses (the difference was that inactivated viruses were used as antigen).[9] IgG subclass Ab responses were determined after immunization with the nanoparticles. Significant levels of IgG1, IgG2b, and IgG3 were detected and the lack of IgG2a reflected only type 2 helper T cells (Th2) responses (Figure S3 in the Supporting Information). Antibody IgG1 generation is usually associated with Th2 responses, whereas high level of IgG2a, are thought to reflect Th1 responses. Th2 response mediates cellular immunity to viruses or intracellular bacteria. A similar response pattern has been found for the non-self-adjuvanting vaccine candidate that is based on the diphtheria toxoid and acts against GAS.[19]

As J14 was a chimera of the streptococcal M protein derived peptide and yeast GCN4-protein sequences, the assessment of whether the antibody response elicited by nanoparticles was indeed directed to the M protein derived sequences was essential.[20] Therefore, we evaluated the ability of the antibodies generated above to bind to α-helical 20-mer epitope p145 and found significant levels of p145-specific antibodies after subcutaneous immunization with **2** (Figure 2b). This observation implies that antibodies elicited by the nanoparticles should be able to recognize the GAS M protein.

The strategy developed here offers an attractive alternative to conventional vaccine approaches. The resulting nanoparticles consist of a peripheral antigenic epitope layer conjugated to a polymer core, and are both self-adjuvanting and produce a strong immune response to the GAS M protein. The nanoparticles formed through the self-assembly of well-defined J14–polymer dendrimers was of a size (20 nm) that allowed possible maximum exposure to the immune system. Our dendrimer–nanoparticles vaccine approach should be readily acquiescent to other pathogenic organisms apart from GAS, and may prove particularly useful for the design of vaccines against infectious diseases known to stimulate an autoimmune response in a host.

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