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Engineered hybrid spider silk particles as delivery system for peptide vaccines

Matthias Lucke [a, e, 1], Inès Mottas [b, c, h, 1], Tina Herbst [b], Christian Hotz [b], Lin Römer [f], Martina Schierling [d], Heike M. Herold [d], Ute Slotta [f], Thibaud Spinetti [b], Thomas Scheibel [d, 2], Gerhard Winter [a, 2], Carole Bourquin [b, c, g, h, **, 2], Julia Engert [a, *, 2]

[a] Department of Pharmacy, Pharmaceutical Technology & Biopharmaceutics, Ludwig-Maximilians-University Munich, Butenandtstrasse 5, 81377 Munich, Germany
[b] Department of Medicine, Faculty of Science, University of Fribourg, Chemin Du Musée 5, 1700 Fribourg, Switzerland
[c] Ecole de Pharmacie Genève-Lausanne, University of Geneva, Rue Michel-Servet 1, 1211 Geneva, Switzerland
[d] University of Bayreuth, Faculty of Engineering Science, Chair for Biomaterials, Universitätsstrasse 30, 95440 Bayreuth, Germany
[e] Coriolis Pharma, Fraunhoferstrasse 18B, 82152 Planegg/Martinsried, Germany
[f] AMSilk GmbH, Am Klopferspitz 19, 82152 Planegg/Martinsried, Germany
[g] Department of Anesthesiology, Pharmacology and Intensive Care, Faculty of Medicine, University of Geneva, Rue Michel-Servet 1, 1211, Geneva, Switzerland
[h] Ecolede Pharmacie Genève-Lausanne, University of Lausanne, Rue Michel-Servet 1, 1211 Geneva, Switzerland

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ABSTRACT

The generation of strong T-cell immunity is one of the main challenges for the development of successful vaccines against cancer and major infectious diseases. Here we have engineered spider silk particles as delivery system for a peptide-based vaccination that leads to effective priming of cytotoxic T-cells. The recombinant spider silk protein eADF4(C16) was fused to the antigenic peptide from ovalbumin, either without linker or with a cathepsin cleavable peptide linker. Particles prepared from the hybrid proteins were taken up by dendritic cells, which are essential for T-cell priming, and successfully activated cytotoxic T-cells, without signs of immunotoxicity or unspecific immunostimulatory activity. Upon subcutaneous injection in mice, the particles were taken up by dendritic cells and accumulated in the lymph nodes, where immune responses are generated. Particles from hybrid proteins containing a cathepsin-cleavable linker induced a strong antigen-specific proliferation of cytotoxic T-cells in vivo, even in the absence of a vaccine adjuvant. We thus demonstrate the efficacy of a new vaccine strategy using a protein-based all-in-one vaccination system, where spider silk particles serve as carriers with an incorporated peptide antigen. Our study further suggests that engineered spider silk-based vaccines are extremely stable, easy to manufacture, and readily customizable.

1. Introduction

Vaccination prevents the spread of many deadly infectious diseases, and it is considered to be one of the most effective interventions for global health ever developed [1]. The protective effect of vaccines is essentially due to B lymphocyte production of neutralizing antibodies against infectious agents or toxins. Although in many infectious diseases this humoral immunity is of major importance, in some cases cellular immunity is essential for protection [2]. Indeed, cytotoxic T-cells, the main effector cells of cellular immunity, are required for the development of successful immune responses against chronic infections such as HIV, malaria

* Corresponding author. Department of Pharmacy, Pharmaceutical Technology & Biopharmaceutics, Ludwig-Maximilians-University Munich, Butenandtstrasse 5, 81377 Munich, Germany
** Corresponding author. Ecole de Pharmacie Genève-Lausanne, University of Geneva, Rue Michel-Servet 1, 1211 Geneva, Switzerland.
E-mail addresses: carole.bourquin@unige.ch (C. Bourquin), julia.engert@cup.uni-muenchen.de (J. Engert).
[1] First authors equal contribution: Matthias Lucke and Inès Mottas contributed equally to this work.
[2] Senior authors equal contribution: Thomas Scheibel, Gerhard Winter, Carole Bourquin and Julia Engert contributed equally to this work.

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or tuberculosis [3]. Furthermore, recent studies have shown that T-cell-mediated immunity can lead to cancer regression in patients, even in the case of advanced disease [4]. Unfortunately, most current vaccination strategies do not induce effective cytotoxic T-cell responses and the development of vaccines that promote cellular immunity thus remains a major challenge [5].

The initiation of cytotoxic T-cell responses requires the priming of naïve $CD8^+$ T-cells by professional antigen-presenting cells such as dendritic cells [6,7]. Dendritic cells are located in many tissues and constantly sample their surroundings. When these cells encounter microbes or particulate matter, or indeed vaccination-delivered antigens, these are engulfed and processed in order to cross-present antigenic peptides to T-cells via major histocompatibility complex class I molecules (MHC I) [8,9]. To induce effective immunity, immature dendritic cells must be activated by specific maturation signals [10]. For this reason, in addition to microbial antigens, most vaccines include adjuvants that stimulate dendritic cell maturation and thus the generation of cytotoxic T-cell immunity [11,12]. The maturation of dendritic cells occurs during their migration from peripheral tissues to the lymph nodes [13]. In the lymph nodes, $CD8^+$ T-cells are primed by direct contact with mature dendritic cells presenting their cognate antigen [14]. Primed T-cells proliferate in the lymph nodes and differentiate to effector cytotoxic T-cells that patrol the body to kill infected or tumoral cells.

The elucidation of the amino acid sequences of microbial or tumor-associated antigens has led to the development of peptide vaccination [15]. Vaccines comprised of antigenic peptides instead of full-length proteins activate only the cytotoxic T-cells that recognize disease-specific epitopes, thus limiting the risk of unspecific autoimmune toxicity. However, peptide vaccination has shown limited success due to several factors. First, peptides are rapidly degraded by proteases: the MelanA/MART-1 tumor antigenic peptide for instance is degraded in 22 s in plasma [16]. Second, peptides can bind to MHC I not only on antigen-presenting cells but on all nucleated cells, thus promoting tolerance rather than immunity [17,18]. Furthermore, to be effective the vaccine peptides must form a stable complex with MHC I molecules during dendritic cell migration to the lymph node [19,20], which takes approximatively 18 h [21]. To meet these challenges, antigens can be protected by particulate carriers. Particle delivery systems can limit peptide degradation, improve uptake of antigen by dendritic cells [22] and ensure that the antigen is processed intracellularly before loading onto MHC I [23].

For the design of particle-based delivery systems, antigen can be coupled to the particles by different methods [24]. The antigen may be adsorbed to the surface of the particle, as is the case with aluminum-based formulations [25]. However, binding by non-covalent interactions may result in rapid antigen release from the carrier upon changes in pH. The antigen can also be encapsulated in a polymer matrix during manufacturing, but this may damage protein-based antigens due to the relatively harsh conditions commonly applied during particle preparation [26]. Chemical conjugation of the antigen to the carrier bears the risk of losing certain epitopes during conjugation [27]. To circumvent these difficulties, we chose to use a protein-based carrier and directly incorporated the antigenic peptide into the sequence of the carrier protein derived from spider silk. Spider silk protein is a promising material, because previous studies demonstrated that the protein itself is poorly immunogenic and does not cause inflammatory reactions in rats [28,29]. Indeed, in addition to their clear advantage in terms of biocompatibility and biodegradability, spider silk particles allow for solvent-free synthesis and steam sterilization [30,31].

2. Materials and methods

2.1. Preparation of spider silk hybrid proteins

eADF4(C16) is based on 16 repeats of the consensus sequence of spidroin ADF4 of the European garden spider (Araneus diadematus) (C-module: GSSAAAAAAAASGPGGY GPENQGPSGPG-GYGPGGPG), and a T7-tag fused to the amino terminus for detection purposes [32]. $OVA_{257\text{-}264}$ peptide (SIINFEKL) was fused to the eADF4(C16) by either using a cathepsin B cleavable linker (GFLG) or a cathepsin S cleavable linker (PMGLP). Fusions were made using tags and DNA sequences of the tags were inserted into the cloning vector pCS of eADF4(C16) by seamless cloning as described previously [31,32]. The resulting constructs and the modified proteins are shown in Fig. S1. Successful cloning was confirmed by sequencing.

2.2. Preparation of spider silk hybrid particles

Preparation of spider silk hybrid particles was performed using the well-established preparation technique using a micromixing system as described before [33]. Briefly, spider silk protein powders were dissolved in a 6 M guanidinium thiocyanate solution and subsequently dialyzed against a 10 mM Tris/HCl solution. The dialyzed solution was 0.2 μm filtered before particle preparation. The particle preparation was subsequently conducted by mixing pretempered spider silk protein solution (1 mg/ml) with either a pretempered 2 M potassium phosphate solution or 2–4 M ammonium sulfate solution. Resulting spider silk particles were washed with highly purified water (HPW) three times followed by a two-minute ultrasonication treatment.

2.2.1. Preparation of spider silk hybrid particles for in vitro and in vivo studies

The before mentioned particle preparation process was slightly adjusted for particles used during in vitro and in vivo experiments. Prior to dissolution in 6 M guanidinium thiocyanate the spider silk powder was suspended in HPW and steam sterilized for 15 min at 121 °C. In addition, chemicals with low endotoxin levels were used throughout the whole preparation process. Endotoxin content of the protein solution was determined using an Endosafe-PTS reader (Charles River Laboratories, Wilmington, USA) after a 20–40-fold dilution with HPW.

2.3. Particle sterilization

Suspensions of spider silk particles were sterilized by autoclave treatment in a GTA 50 autoclave (Fritz Gössner, Hamburg, Germany) as described before [34]. All spider silk particles were suspended in highly purified water at a concentration of 1 mg/ml. Sterilization was performed for 15 min at 121 °C.

2.4. Fluorescent labelling

Labelling of spider silk protein with fluorescein isothiocyanate (FITC) was performed based on the published method by Spieß et al. using the terminal amine group of the spider silk protein [30]. For the preparation of fluorescently labeled particles used for in vivo studies, an endotoxin-free 20 mM HEPES solution instead of a 10 mM Tris/HCl solution was used for dialysis to facilitate the coupling of FITC. After dialysis, a 20-fold molar excess of FITC (dissolved in DMSO) was added slowly to the spider silk solution followed by a 3 h incubation in the dark. The FITC-coupled spider silk protein solution was used for particle preparation as described before. The fluorescent labelling of particles used for in vitro studies