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Two B-cell epitope vaccines based on uPA effectively inhibit fertility in male mice

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ABSTRACT

uPA, a trypsin-like serine protease, was found to take active part in male reproduction. Our previous work had demonstrated the antifertility effects of its full length protein immunization, but with immune tolerance and other latent side effects. Here we discovered two effective B-cell epitopes of uPA for male contraception in growth factor-like domain and kringle domain respectively. Together with carrier protein, immunization of these two epitope peptides could induce high titers of specific antibodies in male mice. Significant reduction of fertility was observed in these two groups in mating trial without evident systemic illness or abnormal mating behavior. Epididymal sperms of immunized males exhibited impaired progressive motility and ability to fertilize eggs in vitro. The immunization of another predicted epitope in serine protease domain and the control groups showed no similar positive results. Importantly, T cells were not activated after the challenge of these B-cell epitopes itself, which suggests that these vaccines do not induce cell-mediated autoimmunity. Taken together, our study discovered two uPA B-cell epitopes as novel targets for male immunocontraception with minimum side effects. Considering their high identity with human uPA protein, these two epitope vaccines hold great promise to be developed for man use in the future.

1. Introduction

The world population is exploding at a tremendous rate and there is an urgent call for more effective, safe, reversible, acceptable and inexpensive contraceptive methods. However, the now available methods are rather limited, especially for men. The now practiced options are limited to condoms, early withdraw and vasectomy [1]. Immunocontraception is an ideal alternative for male contraception [2,3]. It can interrupt multiple reproduction processes like gamete production, function and outcome [4]. Antifertility vaccine can fulfill most of the qualities of a desirable contraceptive [5]. And it does not require surgical intervention nor affects sexual pleasure as the traditional ways do. Thus immunocontraception is considered as one of the most promising contraceptive methods to be developed.

Urokinase type plasminogen activator (uPA) belongs to the plasminogen activator family [6]. It binds to its receptor uPAR on cell surface and activates plasminogen or growth factor for the subsequent proteolysis process. uPA was found to take active part in multiple processes of male reproduction like spermatogenesis [7], sperm maturation and venting [8]. And it can also enhance sperm motility [6], induce acrosome reaction [9] and promote fertilization [10] in vitro (Fig. 1). In our preliminary exploration, we have found that subcutaneous injection of full-length human uPA protein to male mice could effectively reduce their fertility [11]. All these research findings suggest that uPA might serve as a promising target for immunocontraception.

However, immunization with full-length protein of uPA may cause some potential side effects or induce immune tolerance when dosage increases. Compared to traditional vaccines, B-epitope vaccines offer us a better choice since they possess many advantages like stable chemical property, high immune specificity and low toxicity [12]. Epitope vaccine inoculation is expected to induce high titers of oligoclonal response against a few epitopes of interest rather than polyclonal response [13,14]. Thus the latent side effects are reduced. In fact, in our previous full-length uPA protein immunization experiment, the problem of immune tolerance occurred when the uPA dosage reached a high level [11],

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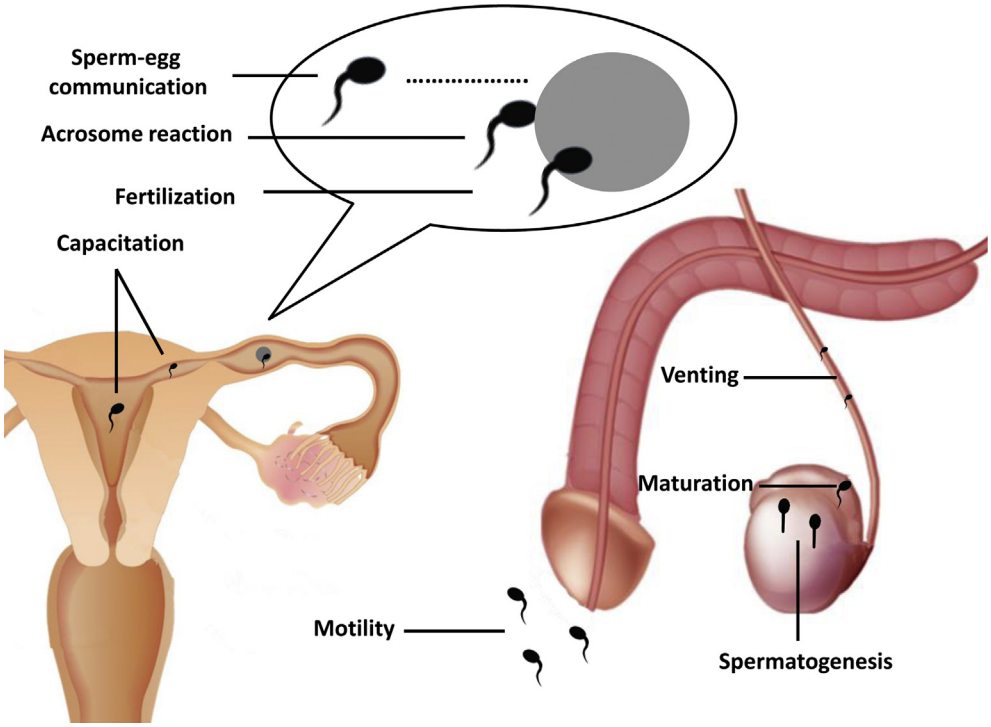

Fig. 1. The main target sites of uPA in male and female reproductive organ. Involved processes include spermatogenesis, sperm maturation, venting, motility in male genital organs, and sperm capacitation, sperm-egg communication, acrosome reaction and fertilization in female.

revealing the shortcoming of full-length protein strategy in vaccine development.

On the base of our previous full-length uPA vaccine, we sought to develop a much safer and more efficient B-epitope vaccine of uPA. So we constructed three B-epitope vaccines accompanied with carrier protein based on the structure and function of mouse uPA, and evaluated their contraceptive effect on male mice. We aimed to develop a novel B-epitope vaccine targeting uPA with ideal effects and minimum side effects for male contraception.

2. Materials and methods

2.1. Animals

Male and female BALB/C mice (aged 7–8 weeks) were obtained from Hubei Provincial Center for Disease Control and Prevention in China. Animals were maintained in a temperature-controlled room with a 12 h light and 12 h dark cycle. All animal experimental procedures were performed in accordance with the NIH Guiding Principles in the Care and Use of Animals and were approved by the Reproductive Medicine Review Board at Tongji Medical College.

2.2. Peptide synthesis

BCEPRED online (http://www.imtech.res.in/raghava/bcepred/) was used to predict the B epitopes of mouse uPA (NP_032899.1) based on a combination of the physico-chemical properties (flexibility, exposed surface, antigenic propensity and accessibility) of the sequence. Three epitopes were predicted and named by us as GF34, K121, SP231. They are (CQNGGVCVSYKYFSR, 34–48), a 15-mer peptide in growth factor-like domain (GF34); (NYCRNPDNQKRPWCY, 121–135), a 15-mer peptide in kringle domain (K121) and (LPKKENYVVYLGQSK, 231–245), also a 15-mer peptides and located in serine protease domain (SP231). These peptides showed no significant homology with any other mouse sequences. Peptides were synthesized by Shanghai Biotech Bioscience and Technology Co., Ltd., China. The purity of all peptides was above 95% and each peptide was coupled with an equal quantity of carrier protein of keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA). KLH and BSA conjugated peptides were used in mice immunization and ELISA respectively.

2.3. Treatment of animals

Male mice were randomly divided into five groups as below:

1. Phosphate-buffered saline (PBS) control group: mice in this group were handled with a subcutaneous injection with 200 μl PBS for each time point.
2. Adjuvant control group: mice were injected with 100 μl PBS mixed with 100 μl Freund's adjuvant (Sigma-Aldrich Co., St. Louis, MO, USA) and KLH.
3. GF34 group: mixed injection solution of 100 μl PBS and 100 μg KLH coupled GF34 immunogen emulsified in 100 μl Freund's adjuvant.
4. K121 group: K121 immunogen in the same emulsification and dilution system as of the GF34 group.
5. SP231 group: SP231 immunogen in the same injection solution as above.

Each mouse was subcutaneous injected once a week for four times. The first injection of antigen was emulsified with Freund complete adjuvant. For the subsequent three booster injections, Freund incomplete adjuvant was used. The schedule of immunization, bleeding, mating and killing of mice were shown in Fig. 3A. Additionally, five mice from each group were euthanized on day 28, and their spleens were excised for T-cell proliferation and ELISPOT assays. The other mice were used for mating and bleeding experiments.