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Peptide vaccines against cancer, infectious diseases, and conception

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1. ABSTRACT

The concept of peptide vaccines is based on identification and chemical synthesis of B-cell and T-cell epitopes which are immunodominant and can induce specific immune responses. B-cell epitope of a target molecule can be coupled to a promiscuous T-cell epitope to make it immunogenic. Our increased understanding of antigen recognition at molecular level has resulted in the development of rationally designed peptide vaccines. The relative ease of construction and production, chemical stability, and lack of oncogenic or infectious potential has made the peptides attractive vaccine candidates. However, several obstacles limit the widespread usefulness of peptide vaccines. These include their low immunogenicity, need for a better adjuvant and carrier, and reliable and simple assays to measure T-cell response. Nonetheless, current efforts are defying these limitations and many promising discoveries are making their way to improve this approach. The peptide vaccines against various cancers have undergone phase I and phase II clinical trials with successful immunological clinical outcome. The peptide vaccination is being examined both for palliative and prophylactic immunotherapy. The current status of many peptide vaccines which are being developed against cancer, infectious diseases, and conception is discussed in this review.

2. INTRODUCTION

Vaccines are one of the most effective and successful prophylactic and palliative modalities for infectious and non-infectious diseases. With the advent of vaccines, there has been a worldwide decrease in several infectious diseases (1). The vaccination programs have been successful in worldwide eradication of smallpox, and

have decreased the incidence of several diseases. The poliomyelitis has gone down by almost 100%, diphtheria, measles and rubella by 99% and whooping cough has decreased by 97% (1). For several diseases either the vaccines have not been developed, or the existing vaccines are not efficacious for complete eradication. Several novel and innovative strategies are being explored to construct new vaccines, and to improve the efficacy of existing vaccines. The development of synthetic peptide vaccines is one of these exciting strategies. Lately, the synthetic peptide vaccines have drawn considerable attraction because of the significant advances in peptide synthesis, proteomics, 2 dimensional gel-electrophoresis coupled with matrix assisted laser desorption ionization-time of flight-mass spectrometry (2D gel-MALDI-TOF-MS), and liquid chromatography-mass spectrometry (LC-MS), and peptide phage display technology. The peptide epitopes can now easily be delineated, sequenced, and large quantities of pure, potent and highly specific peptides can be synthesized to produce a vaccine that can raise an immune response to attenuate the effect of a disease. Various synthetic adjuvants and carriers can be incorporated into peptide vaccine formulations to enhance the immunogenicity. The solubility and stability of the peptides can be enhanced by incorporation of carbohydrates, and multiple peptide epitopes can be assembled into a single vaccine formulation by chemical ligation techniques (2). Vaccines are not only important for infectious diseases, but are also being constructed for non-infectious diseases/conditions, such as cancer and conception. The aim of the present article is to review the status of synthetic peptide vaccines that have been tried for various cancer conditions, infectious diseases, and contraceptive purposes. The objective is to learn the lessons from the synthetic peptide vaccine technologies that have been successfully tried against various cancer/infections/conditions in humans, and incorporate them for the development of synthetic peptide contraceptive vaccines.

Recently, several cancers and their related deaths are substantially increasing. In fact, over 10 million people are diagnosed with cancer, and more than 6 million people die due to cancer related deaths every year worldwide (3). Even with the refined surgical techniques/combination of chemotherapeutic agents/less invasive radiation procedures the mortality of cancer patients continues. These therapies damage healthy cells and cause serious side effects such as nausea, vomiting, alopecia, and increase susceptibility to additional tumors (4). For these reasons, it is imperative to develop alternative approaches for the treatment and management of cancer patients (5). One such approach currently being investigated is the administration of peptide based vaccines, in a form of immunotherapy aimed at activating the patients' immune system to recognize and defeat the malignant "foreign" tumors (6). Tumor cells present peptides that are unique and aberrant on cell surface with respect to the major histocompatibility complex (MHC). These unique tumor associated antigens make it possible to raise specific immune responses that can target tumors, but spare the normal tissues (7-12). The peptide based vaccines have a number of advantages over other forms of anti-tumor immunotherapy because peptides are chemically well-defined and relatively stable molecules. They are easy and comparatively less expensive to manufacture, and there are no infectious agents and contaminating substances involved in manufacturing. Thus, they are devoid of oncogenic potential. Different molecules and T-cell epitopes can be linked with synthetic peptides to enhance their immunogenicity. Currently, there are several peptide vaccines in clinical trials for cancer immunotherapy. These are discussed below.

3. DISCUSSION

3.1. Peptide Vaccines for Various Cancers

Various vaccines based upon several peptides have been tried for cancer immunotherapy. The notable peptide vaccines that have undergone phase I/II/III clinical trials are discussed below.

3.1.1. Heregulin (HER)-2/neu peptide

HER-2/neu is a 185 kD transmembrane protein of the epidermal growth factor receptor family (13). HER-2/neu is weakly expressed on the epithelial surface of normal tissue, but is over-expressed on the surface of tumor cells of breast, ovary and lung. In breast carcinoma, HER-2/neu is over-expressed in ~30% of patients and hence it is an interesting target for immunotherapy. "Herceptin", a monoclonal antibody to HER-2/neu, has been licensed for the treatment of breast cancer (14-16).

Recently, a study was conducted to determine whether HER-2/neu cytotoxic T-lymphocytes (CTL) immunity could be elicited in the absence of $CD4^+$ T-cell help (17). A total of six human-leukocyte antigen (HLA-A2) patients with HER-2/neu over-expressed tumors and stage III or IV breast or ovarian cancer were recruited. They received a monthly intradermal injection for six months of HER-2/neu peptide p369-377 mixed with granulocyte-macrophage-colony stimulating factor (GM-CSF) as an adjuvant. The p369-377 is an immunodominant, HLA class I binding peptide that is part of the extra-cellular domain of HER-2/neu protein. After immunization, CTL response was detected which subsided after five months. Along with the previous study (18), these findings indicate that $CD4^+$ T-cell help is required for long lasting immunity.

HER-2/neu peptide has undergone two clinical trials in which the safety, toxicity and efficacy were examined. Patients were immunized with one of two helper T-cell vaccines that were targeted against the extra-cellular or intra-cellular domains, respectively. The patients included in this study have been treated for stage III or IV breast, lung or ovarian cancers in which HER-2/neu was over-expressed. Six of the eight patients developed HER-2/neu protein specific $CD4^+$ T-cell responses. No toxicity was noted against tissues that express basal levels of HER-2/neu such as skin, gastrointestinal tract and epithelium of lung.

Another approach that has been tried for vaccination against HER-2/neu is peptide pulsing of autologous dendritic cells (DCs). A phase I clinical trial was conducted on gastric cancer patients using autologous

DCs pulsed with the HER-2/neu immunodominant peptide, p369-377 (19). A total of nine HLA A2 patients having HER-2/neu over-expressing tumors were given four intradermal injections at two-week intervals. Peripheral blood mononuclear cells (PBMCs) were collected before and four weeks after the last injection and were evaluated for IFN -γ release. After vaccination, six of the nine patients demonstrated peptide-specific CTL activity. The delay-type hypersensitivity (DTH) responses were apparent in three patients. In addition to having peptide specific T-cell responses, two patients also demonstrated clinical response in cancer improvement.

3.1.2. Mucin-1 (MUC-1) peptide

MUC-1 is a mucin family glycoprotein which is composed of a polypeptide core containing multiple tandem repeats of a 20 amino acid sequence known as variable number of tandem repeats (VNTR). Mucins are highly glycosylated by O-linked carbohydrate side chains (20) produced by cells of epithelial origin. In cancer, there is abnormal MUC-1 glycosylation that has fewer carbohydrate side chains. This causes an increase of MUC-1 peptide epitopes to the immune system (21). These epitopes can induce both an antibody and cytotoxic T-lymphocyte response.

Recently, a 106 a.a. peptide comprising five VNTR motifs-conjugated to keyhole limpet hemocyanin (KLH) was injected with QS-21 adjuvant to nine patients having II, III or VI stage breast cancer (22). After vaccination, seven patients developed strong IgG and IgM antibodies directed against MUC-1 peptide. There was no evidence of T-cell activation, and in two patients the disease reoccurred (22).

To enhance a MUC-1-specific CTL response, MUC-1 peptide was conjugated to mannan. Mannan acts as a delivery system to target MUC-1 peptides to the major histocompatibility (MHC)-I pathway, enhancing their presentation to CTLs (23). In this study, the effect of cyclophosphamide and intraperitoneal (i.p.) route of administration was assessed. Forty-one patients with metastatic or locally advance breast or colon cancer received increasing doses (1-300 μg) of MUC-1 fusion protein at weekly intervals for three weeks, and the cyclophosphamide was administered twice week on one and four, respectively. In five patients, the disease stabilized. Approximately 60% of patients demonstrated a high titer of IgG antibody after vaccination. The effects exasperated in patients receiving the vaccine via i.p. route. Only 28 % of patients demonstrated MUC-1 specific T-cell reactivity, with or without the addition of cyclophosphamide (23). The vaccination via i.p. route enhanced the antibody response. The administration of cyclophosphamide had no effect on increasing MUC-1-specific T-cell immunity.

3.1.3. Carcinoembryonic antigen (CEA)

CEA is a glycoprotein not normally expressed in adult tissues but over-expressed in most colorectal, gastric, breast, pancreatic and non-small cell lung cancers. Increased expression of CEA on tumor cells mediate the attachment of tumor cells to normal cells and promotes the metastasis (24). Several immunotherapies against CEA have been developed such as, monoclonal antibody therapy, DNA-based vaccination with or without different viral delivery vehicles, and peptide vaccination (25). Three phase I clinical trials were conducted involving CEA peptides with or with calcitonins, delivered with autologous DCs (26, 27). The immunological evaluation showed no increase in cellular immune reactivity after peptide vaccination (26). Vaccination with peptide on detox PC adjuvant enhanced T-cell, but not the clinical responses (26-28). The epitope enhancement increased the efficacy of a CEA peptide vaccine (29), and immunization with DCs pulsed with a HLA-A-24- presented CEA peptide caused a clinical improvement (30).

3.1.4. Prostate-specific membrane antigen (PSMA)

PSMA is an integral membrane protein that is over-expressed in some prostate carcinoma (31). Its levels are elevated in the serum of patients with advanced prostate cancer (32). A vaccine comprised of dendritic cells pulsed with two PSMA peptides showed promising results in phase II trials (33). Recombinant PSMA has been produced in baculovirus (34).

3.1.5. Human papilloma virus (HPV)

Many tumors have antigens that are coded by viral genome. There are many virally-induced tumors such as B-cell lymphomas related to Epstein-Barr virus (EBV), adult T-cell leukemia associated with human T-lymphocyte-1 (HTL-1), and the cervical carcinoma associated with HPV. More than 90% of human cervical cancer is believed to be caused by HPV (35), and HPV DNA has also been detected in other tumors such as penile and pharyngeal carcinoma (36). HPV carries two oncogenes, E6 and E7 (37). E6 contributes to malignancy by binding and facilitating the degradation of the tumor suppressed protein, P53 (38). E7 contributes to malignancy by binding and inactivating another tumor suppressor protein, Rb (39). Immune response to HPV antigens is generally weak with the exception of E7 protein (40). A dominant epitope of E7 is an effective vaccine candidate against tumors expressing E7 (41).

HPV epitopes binding to HLA molecules have been mapped and used in several peptide vaccines. In initial clinical trials with HPV-16, E7 peptide vaccines were not very successful in inducing CTL or clinical responses (42-44). However, a recent vaccine using two peptides from E7 (12-20 a.a. and 86-93 a.a., respectively) showed increased CTL activity along with some viral clearance in 62% of patients (45).

Although the peptide vaccines have shown some promising results, the Food and Drug Administration (FDA) on June 8, 2006 approved the first vaccine for cervical cancer designated as Gardasil, manufactured by Merck & Co., Inc. This is a bivalent L1 virus-like vaccine, primarily against HPV types 16 and 18, that has shown sustained protective immunity for several years (46).

3.1.6. Ras oncoprotein

Ras is an oncogene (p21G) product which is located on chromosome 11. It is found in normal cells where it is a part of several signal transduction pathways. If a cell-surface receptor is not stimulated by ligand binding, Ras is not activated, and the pathway that causes cell growth is not initiated. In ~30% of human cancers including colorectal and pancreatic carcinomas (47), Ras is mutated and permanently switched on, signaling the cells to continue growing regardless of cell surface receptor activation. The most point mutations are in codons 12 and 61. Mutations in Ras result in the production of an altered p21G protein that is continuously active and may cause cell transformation and tumorigenesis.

Several clinical trials have been carried out to immunize against mutant Ras 13 a.a. peptide with adjuvant (48) or 10 a.a./17 a.a. peptide pulsed on to antigen presenting cells, PBMC or enriched DCs (49). Although no significant clinical responses were seen in these studies, a correlation between specific cytokine response and survival was observed (49). In another study, after intradermal immunization of pancreatic cancer patients with Ras peptide along with GM-CSF as adjuvant, the median survival was longer in those achieving an immune response (50).

3.1.7. Melanoma antigens

Tumors developed by spontaneous remission are the major focus of cancer vaccines and immunotherapy (51, 52). Melanoma has the longest history among all cancers that have been targeted by immunotherapy. Three groups of tumor-associated antigens (TAA) have been examined clinically for vaccine development against melanoma. The melanocyte differentiation antigens (Melan A/ MART-1, GP100, and Tyrosinase) are expressed in melanocytes and melanoma tumors, but are not expressed in tumors of other origins (53-55). There are many reviews on clinical trials using peptide vaccines against melanoma antigens (56-58).

3.2. Peptide Vaccines for Infectious Diseases

Several peptide vaccines have been tried for various infectious diseases. However, they are still in experimental stage in animal models. No peptide vaccine has reached phase I clinical trial in humans, at the present time. The details and stage of development of vaccines against infectious diseases can be found at the WHO website "http://www.who.int/vaccine_research/documents/en/".

3.3. Peptide Vaccines for Contraception

The following peptide vaccines based upon reproductive hormones and gamete antigens have been investigated for immunoregulation of fertility (contraception/birth control).

3.3.1. Gonadotropin releasing hormone (GnRH) peptide

GnRH is a decapeptide secreted by the hypothalamus and activates the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary. FSH and LH regulate gametogenesis and the production of sex hormones (androgens and estrogens). GnRH being a decapeptide requires T-cell epitopes by conjugation with a T-cell carrier to make it immunogenic. Various T-cell carriers have been tried. GnRH conjugate vaccine causes a block in fertility of both male and female animals of several species (59-63). GnRH vaccines affect gonadal function, sexual behavior, feedlot performance, and carcass mass. In males, it has been used as a substitute for castration. Several GnRH vaccines using different adjuvants and carriers have undergone clinical trials in different countries sponsored by various pharmaceutical companies for atrophy of the prostate and for several cancers in humans (64-66).

3.3.2. Human chorionic gonadotropin (hCG) peptide

hCG is pregnancy associated hormone, with two subunits (α and β). The α subunit is shared with three other pituitary hormones (LH, FSH, and thyroid-stimulating hormone [TSH]), and β subunit is specific. For vaccine development, two approaches were investigated, use the whole β subunit of hCG (β-hCG) as an immunogen or 37 a.a. carboxyterminal peptide (CTP) of β-hCG. The 37 a.a. CTP sequence is specific to β-hCG without any homology with β-LH. The phase I clinical trials, conducted in women, using both the whole β-hCG vaccine (67) and the CTP vaccine (68) did not show any adverse effects. Both these vaccines produced low titers with the CTP vaccine being the weaker of the two. The whole β-hCG vaccine has also undergone phase II clinical trials in three major hospitals in India. Fertile and sexually active women (20-35 years) were enrolled. The women with antibody titers ≥ 50 ng/mL (hCG bioneutralisation capacity) were protected from pregnancy (69). The antibody response declined with time and fertility was regained when titers fell below 35 ng/mL. CTP based vaccine has not undergone phase II clinical trials because of its poor immunogenicity.

3.3.3. Oocyte zona pellucida (ZP)

ZP is an extracellular matrix that surrounds a mammalian oocyte and pre-implantation embryo (70, 71). Sperm must recognize, attach, acrosome react and pass through the ZP in order to fertilize an egg. ZP represents interesting targets for contraception. It consists of four developmentally regulated, evolutionarily conserved and somewhat gamete-specific glycoproteins, namely ZP1, ZP2, ZP3 and ZP4, which have essential role in fertilization. Of the four glycoproteins, ZP3 is the primary molecule involved in sperm recognition, binding, and induction of acrosome reaction in several species. Mice that are homozygous for an insertional mutation in ZP3 gene lack ZP in the oocyte, and are infertile (72).

Antibodies to ZP3 or its peptide fragments block fertilization (73, 74). Active immunization of female animals of various species, including primates, with ZP3 or its peptide fragments induces infertility (75-79). However, immunization with the whole ZP3 molecule generally causes irreversible oophoritis with loss of primordial follicles and decrease of estrogens, leading to premature ovarian failure (POF). The recent research in several laboratories is focused on delineating B-cell epitopes of various ZP proteins that are devoid of T-cell reactivity, and thus can be used in vaccine formulations that do not cause ovarian pathology. Several B-cells epitopes of ZP3 have

Table 1. Immunobiological parameters of zona pellucida peptides

Peptide epitope	Carrier used[1]	Species tested	Fertility effect	Reference
Mouse ZP2(121-140a.a.)	--	Mouse	Reduced fertility without oophoritis	80
Mouse ZP3 (335-342 a.a.)	--	Mouse	Reduced fertility without oophoritis	84
Mouse ZP3 (328-342 a.a.)	KLH	Wild mouse	Reduced fertility	88
Human ZP3 (328-341 a.a.)	CSP	Mouse	Reduced in vitro human sperm-oocyte binding	89
Human ZPA (50-67 a.a.)	DT	Rabbit	Reduced in vitro human sperm-oocyte binding	81
Feline ZPB (130-149, 175-193 a.a.)	BSA	Cat	Reduced in vitro human sperm-oocyte binding	83
Porcine ZP1(79-130 a.a.)	--	White-tailed deer	Reduced fertility	87
Marmoset ZP3 (301-320 a.a.)	TT	Marmoset monkey	Reduced fertility without oophoritis	85
Bonnet monkey ZPC (324-347 a.a.) ZP3 (334-343 a.a.) ZP1 (58-79,136-153, 212-228, 251-273 a.a.)	DT; CSP	Bonnet monkey	Reduced fertility without oophoritis	82, 86

[1] KLH: Keyhole limpet hemocyanin, CSP: circumsporozoite protein, BSA: Bovine serum albumin, DT: Diphtheria toxoid, TT: Tetanus toxoid

been delineated. Immunization of several species of animals, including sub-human primates with various synthetic ZP B-cell epitopes conjugated to promiscuous T-cell carriers, caused long term reversible infertility without induction of ovarian pathology (80-89) (Table 1). However, the data are equivocal and unconvincing. In some studies, even without the T-cell reactivity in the ovaries, defect in folliculogenesis has been observed. Unless this serious adverse effect of premature ovarian failure is resolved, ZP vaccine is not an acceptable proposition for humans. However, the semi-purified vaccine, prepared from porcine oocyte ZP, designated as pZP, is being successfully used for controlling feral populations of dogs, horses, deer and elephants (90-92).

3.3.4. Sperm peptides

Several sperm peptides have been delineated and are being explored for vaccine development (Table 2). Notable among are discussed below.

3.3.4.1. Fertilization antigen (FA)-1 peptide

The FA-1 antigen is a sperm-specific surface molecule that is evolutionarily conserved on sperm of various mammalian species including humans. It is localized on the post-acrosomal, mid-piece, and tail regions of the sperm cell (93). Antibodies to FA-1 antigen inhibit human sperm-zona interaction, and also block human sperm capacitation/acrosome reaction by inhibiting tyrosine phosphorylation (94). The cDNAs encoding for mouse and human FA-1 have been cloned and sequenced (95, 96). Vaccination of female mice with recombinant FA-1 antigen causes a long-term reversible contraception by raising sperm-specific immune response (97). FA-1 is also involved in human immunoinfertility in both men and women.

3.3.4.2. YLP_{12} peptide

YLP_{12}, YLPVGGLRRIGG, is a dodecamer peptide sequence present on human and murine sperm, which is involved in binding to oocyte ZP3 (98). The synthetic YLP_{12} significantly inhibits human sperm-human ZP binding, and is involved in human immunoinfertility (99). A vaccine was prepared by conjugating YLP_{12} peptide with recombinant cholera toxin B subunit (rCTB) (100) that provided the T-cell epitopes. Vaccination of female mice with the YLP_{12}-rCTB conjugate by various routes (intranasal/intramuscular) induced a contraceptive state resulting in a significant reduction in litter size (overall up to 71%). Antibody reactivity was monitored biweekly and by days 305-322 completely disappeared from serum and vaginal washings of the vaccinated animals. Upon mating the antibody-free animals delivered a normal litter size, indicating regain of fertility (Table 2).

3.3.4.3. Rabbit sperm autoantigen (RSA)/Sp17 peptide

P10G peptide was derived from a sequence of RSA. RSA is comprised of a low molecular weight (~14-17 kD) group of four sperm proteins that function as lectin-like zona binding proteins (101). These are present on the surface of spermatozoa and its antibodies inhibit fertilization. Antibodies that bind to RSA also bind to mouse sperm. The female mice immunized with P10G conjugated with KLH showed a reduction in fertility (102) (Table 2).

A9D is a chimeric peptide derived from the protein Sp17. Sp17 is a 17-kD protein, and a member of the RSA group (103). It is encoded by two mRNAs of 0.9 and 1.1 kb, respectively. Immunization with A9D peptide collinearly synthesized with a promiscuous T-cell epitope of RNAse caused a reduction in fertility of female mice (104) (Table 2).

3.3.4.4. Sp56 peptide

Sp56 peptide (438-454 a.a.) is derived from the recombinant mouse Sp56 protein. The Sp56 cDNA and its reduced amino acid sequence suggest that it is a member of a superfamily of protein receptors. It is specifically expressed in testis and on sperm (105). Immunization of female mice with recombinant mouse Sp56, and its peptide conjugated with KLH caused a reduction in fertility (106, 107) (Table 2).

3.3.4.5. YWK-II peptide

YAL-198, YAL-201, YAL-212 are peptides that were derived from the extracellular domain of YWK-II, a spermatozoa protein. The cDNA encoding YWK-II is 1.8 kB and was isolated from rat testis expression library using a monoclonal antibody that causes sperm agglutination (108). This protein is localized on sperm head and synthesized during spermatogenesis. Immunization of female rats with various peptides causes a reduction in fertility (109) (Table 2).

Table 2. Immunobiological parameters of sperm peptides

Peptide	a.a. (n)[1]	Sequence	Carrier[2]	Strain	Fertility reduction	Reference
A. Mouse						
mFA-1_{2-19}	18	TEADVNPKPIPSQMPTSP	--	--	--	95,97
mFA-$1_{117-136}$	20	QSIQQSIERLWCRLWPLPFP	--	--	--	95,97
YLP_{12}	12	YLPVGGLRRIGG	rCTB	CD-1	Up to 70%	100
P10G	10	PGGGTLPPSG	KLH	BALB/c	>80%	102
A9D	9	AEWGAKVED	RNAse	CD-1/ BALB/c	>50%	104
SP56	16	YLFGHEENSTEHAMKG	KLH	BALB/c	Up to 80%	106,107
B. Rat						
YAL-198	16	SEEIPPFHPFHPFPSL	BSA	Sprague Dawley	59%	109
YAL-201	24	IPPFHPFHPFPSLSENEDTQPELY	BSA	Sprague Dawley	33%	109
YAL-212	32	SSISENPVDVRVSSEESEEIPPFHPFHPFPSL	BSA	Sprague Dawley	16%	109
SMP-229	25	SFEKAMRFLTLPSEDCLMEFGGSS	MAP	Sprague Dawley	25%	111
SMP-230	28	MRISVSEGGSSGLFFSRAFSGVLNVEEV	MAP	Sprague Dawley	83%	111
C. Primates						
LDH-C_4-bC5-19	15	EQLIKKLIEDDKNSQ	TT	Baboon	62%	113
LDH-C_4-bC5-19	15	EQLIKKLIEDDKNSQ	TT	Cynomologous monkey	0%	114

[1] a.a. (n): Number of amino acids, [2]rCTB: recombinant cholera toxin binding subunit, KLH: keyhole limpet hemocyanin, RNAse: ribonuclease, BSA: bovine serum albumin, MAP: multiple antigen peptide, TT: tetanus toxoid

3.3.4.6. rSMP-B peptide

SMP-229 and SMP-230 are peptides that belong to the extracellular domain of the sperm protein, rSMP-B. The cDNA encoding this protein was obtained from rat testis expression library. It is a 2.0 kB cDNA with a reading frame of 438 bp, and coding for a peptide of 146 a.a. (110). Antibodies to rSMP-B inhibit fertilization in vitro. rSMP-B is localized on midpiece and tail of sperm. Even though both female and male rats were immunized with these peptides, only female rats experienced a reduction in fertility (111) (Table 2).

3.3.4.7. LDH-C_4 peptide

LDH-C_4 is a testis-specific isozyme of LDH (112). cDNA encoding LDH-C_4 has been cloned and sequenced. Immunization of female mice with the whole cognate antigen caused a reduction in fertility. LDH-C_4-bC5-19 peptide of this molecule conjugated with a promiscuous synthetic epitope of tetanus toxoid (TT) reduced fertility of female baboons (113). However, a study by another group reported no effect on fertility of female monkeys (Cynomolgus macaque) after immunization with the same peptide vaccine (114) (Table 2). The reason for this discrepancy is unclear.

3.3.4.8. Multi-sperm peptides combination vaccine

No single sperm antigen/peptide has caused a complete block in fertility in the mouse model. To test the hypothesis whether vaccination with more than one peptide could cause a better contraceptive effect, six sperm peptides were selected, namely mFA-1_{2-19}, mFA-$1_{117-136}$, YLP_{12}, P10G, A9D, and SP56 (Table 2). These have been shown to cause > 50% to > 80% reduction in fertility when injected individually. It was envisaged that vaccination with all the six peptides together will enhance the contraceptive efficacy by an additive effect resulting in a complete block in fertility. Six vaccines were prepared by conjugating the six synthetic peptides with the recombinant binding subunit of cholera toxin. Female mice were immunized intramuscularly with all the six peptide vaccines (115). Each animal received a total of five injections at 2- to 3- week interval of all of the six vaccines with each vaccine injected at a separate site. After vaccination with the six peptides, there was an overall 45% reduction compared to controls. Several mice produced antibodies against these peptides in the serum and the genital tract, but the titers were low and many animals did not respond to several peptides. No animal produced antibodies to all the six peptides. When the antibody titers against all the six peptides disappeared after >10 months from circulation and genital tract, all the animals regained fertility. Even with such low titers there was a significant reduction in fertility after immunization with multi-peptide vaccines. These findings are encouraging and suggest further studies with multi-epitope vaccines.

4. CONCLUSIONS

The review on peptide vaccines against cancer, infectious diseases, and conception indicate that the vaccination with synthetic peptides is an interesting and viable proposition both for prophylactic and palliative immunotherapy. Several of the peptide vaccines especially against various cancers have undergone phase I and II clinical trials and have shown promising results in immunological as well as clinical responses. No peptide vaccine against any infectious disease or conception has undergone phase I clinical trial in humans. The peptide vaccines are relatively less expensive, easy to manufacture and manipulate, are of defined structure, and being synthetic in nature do not have a problem of batch to batch variation. The major disadvantage of the peptide vaccines is their weak immunogenicity. Several strategies such as epitope enhancement, use of various T-cell epitopes, adjuvants, incorporation of co-stimulatory molecules, ex-vivo-loading into antigen presenting cells are being explored to enhance the immunogenicity and efficacy of the peptide vaccines (116, 117).

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6. REFERENCES

1. Ada, G. Vaccines and vaccination.*N Eng J Med* 345, 1042-53 (2001)
2. Kackson, D.C., Purcell, A. W., Fitzmaurice, C.W., Zeng, W., and Hart, D. N. J. The central role played by peptides in the immune response and the design of peptide-based vaccines against infectious diseases and cancer.*Current Drug Targets* 3, 175-96 (2002)
3. Salit, R. B., Kast, W. M., and Velders, M. P. Inns and outs of clinical trials with peptide-based vaccines.*Front Biosci* 7, e204-13 (2002)
4. Eds, V.T., DeVitra, Jr., Hellman, S., and Rosenberg, S.A. Biologic therapy of cancer. J.B. Lippencott Company, Philadelphia (1995)
5. American Cancer Society and www.cancer.org: Cancer facts and figures 2001 (2001)
6. Kast, W. M. Peptide-based cancer vaccines. Landes Bioscience, Georgetown, TX (2000)
7. Mellaman, I., and Steinman, R. M. Dendritic cells: specialized and regulated antigen-processing machines.*Cell* 106, 255-8 (2001)
8. Brossart, P., and Beven, M. J. Presentation of exogenous protein antigens on major histocompatibility complex class I molecules by dendritic cells: pathway of presentation and regulation by cytokines.*Blood* 90, 1594-9 (1997)
9. Watts, C. The exogenous pathway for antigen presentation on major histocompatibility complex class II and CD1 molecules.*Nature Immunol* 5, 685-92 (2004)
10. Watts, C., and Amigorena, S. Antigen traffic pathways in dendritic cells.*Traffic*1, 312-7 (2000)
11. Ackerman, A. L., and Cresswell, P. Cellular mechanisms governing cross- presentation of exogenous antigens.*Nature Immunol* 5, 678-84 (2004)
12. Coussens, L. M., and Werb, Z. Inflammatory cells and cancer: Think different!*J Exp Med* 193, F23-6 (2001)
13. Disis, M. L., and Cheever, M.A. HER-2/neu oncogenic protein: issues in vaccine development.*Crit Rev Immunol* 18, 37-45 (1998)
14. Baselga, J. L., Norton, J., Albanell, Y. M., and Mendelsohn, J. Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human barest cancer xenografts.*Cancer Res* 58, 2825-31 (1998)
15. Waldman, T. A. Immunotherapy: past, present and feature.*Nat Med* 9, 269-77 (2003)
16. Slamon, D. J., Leyland-Jones B., Shak, S., Fuchs, H., Paton, V., Bajamonde, A., Fleming, T., Eiermann, W., Wolter, J., Pegram, M., Baselga, J., and Norton, L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2.*N Engl J Med* 344, 783-92 (2001)
17. Knutson, K. L., Schiffman, K., Cheever, M.A., and Disis, M.L. Immunization of cancer patients with a HER-2/neu, HLA-A2 peptide, p369-377, results in short- lived peptide-specific immunity.*Clin Cancer Res* 8, 1014-8 (2002)
18. Knutson, K. L., Schiffman, K., and Disis, M. L. Immunization with a HER-2/neu helper peptide vaccine generates HER-2/neu CD8 T-cell immunity in cancer patients.*J Clin Invest* 107, 477-84 (2001)
19. Kono, K., Takahashi, A., Sugai, H., Fujii, H., Choudhury, A. R., Kiessling, R., and Matsumoto, Y. Dendritic cells pulsed with HER-2/neu-derived peptides can induce specific T-cell responses in patients with gastric cancer.*Clin Cancer Res* 8, 3394-400 (2002)
20. Taylor-Papadimitriou, J., Burchell, J., Miles, D. W., and Dalziel, M. MUC1 and cancer.*Biochim Biophys Acta* 1455, 301-13 (1999)
21. Brossart, P., Heinrich, K. S., Stuhler, G., Behnke, L., Reichardt, V. L., Stevanovics, S., Muhm, A., Rammensee, H. G., Kanz, L., and Brugger, W. Identification of HLA-A2-restricted T-cell epitopes derived from the MUC1 tumor antigen for broadly applicable vaccine therapies. *Blood* 93, 4309-17 (1999)
22. Gilewski, T., Adluri, S., Ragupathi, G., Zhang, S., Yao, T. J., Panageas, K., Moynahan, M., Houghton, A., Norton, L., and Livingston, P. O. Vaccination of high-risk breast cancer patients with mucin-1 (MUC1) keyhole limpet hemocyanin conjugate plus QS-21.*Clin Cancer Res* 6, 1693-701 (2000)
23. Karanikas, V. L., Hwang, A., Pearson, J., Ong, C. S., Apostolopoulos, V., Vaughan, H., Xing, P. X., Jamieson, G., Pietersz, G., Tait, B., Broadbent, R., Thynne, G., and McKenzie, I. F. Antibody and T-cell responses of patients with adenocarcinoma immunized with mannan-MUC1 fusion protein.*J Clin Invest*. 1, 2783-92 (1997)
24. Marshall, L. Carcinoembryonic antigen-based vaccine.*Semin Oncol* 30, 30-6 (2003)
25. Hodge, J. W. Carcinoembryonic antigen as a target for cancer vaccines.*Cancer Immunol Immunother* 43, 127-34 (1996)
26. Morse, M. A., Deng, Y., Coleman, D., Hull, S., Kitrell-Fisher, E., Nair, S., Schlom, J., Ryback, M. E., and Lyerly, H. K. A Phase I study of active immunotherapy with carcinoembryonic antigen peptide (CAP-1)-pulsed, autologous human cultured dendritic cells in patients with metastatic malignancies expressing carcinoembryonic antigen.*Clin Cancer Res* 5, 1331-8 (1999)
27. Schott, M. J., Seissler, M., Lettmann, V., Fouxon, W., Scherbaum, A., Feldkamp, J. Immunotherapy for medullary thyroid carcinoma by dendritic cell vaccination.*J Clin Endocrinol Metab* 86, 4965-9 (2001)
28. Arlen, P., Tsang, K. Y., Marshall, J. L., Chen, A., Steinberg, S. M., Poole, D., Hand, P. M., Schlom, J., and Hamilton, J. M. The use of a rapid ELISPOT assay to analyze peptide-specific immune responses in carcinoma patients to peptide vs. recombinant poxvirus vaccines.*Cancer Immunol Immunother* 49, 517-29 (2000)
29. Huarte, E., Sarobe, P., Lu, J., Casares, N., Lasarte, J. J., Dotor, J., Ruiz, M., Prieto, J., Celis, E., and Borras-Cuesta, F. Enhancing immunogenicity of a CTL epitope from carcinoembryonic antigen by selective amino acid replacements.*Clin Cancer Res* 8, 2336-44 (2002)
30. Itoh, T., Ueda, Y., Kawashima, I., Nukaya, I., Fujiwara, H., Fuji, N., Yamashita, T., Yoshimura, T., Okugawa, K., Iwasaki, T., Ideno, M., Takesako, K., Mitsuhashi, M., Orita, K., and Yamagishi, H. Immunotherapy of solid cancer using dendritic cells pulsed with the HLA-A24-restricted peptide of carcinoembryonic antigen.*Cancer Immunol Immunother* 51, 99-106 (2002)
31. Kawakami, M., and Nakayama, J. Enhanced expression of prostate-specific membrane antigen gene in prostate

cancer as revealed by in situ hybridization.*Cancer Res* 57, 2321-4 (1997)
32. Murphy, G. P., Holmes, E. H., Boynton, A. L., Kenny, G. M., Ostenson, R. C., Erickson, S. J., and Barren, R. J. Comparison of prostate specific antigen, prostate specific membrane antigen, and LNCaP-based enzyme-linked immunosorbent assays in prostatic cancer patients and patients with benign prostatic enlargement.*Prostate* 26, 164-8 (1995)
33. Salgaller, M. L., Lodge, P. A., McLean, J. G., Tjoa, B. A., Loftus D. J., Ragde, H., Kenny, G. M., Rogers, M., Boyton, A. L., and Murphy, G. P. Report of immune monitoring of prostate cancer patients undergoing T-cell therapy using dendritic cells pulsed with HLA-A2-specific peptides from prostate-specific membrane antigen (PSMA).*Prostate* 35, 144-51 (1998)
34. Lodge, P. A., Childs, R. A., Monahan, S. J., McLean J. G., Sehgal, A., Boynton, A. L., Salgaller, M. L., and Murphy, G. P. Expression and purification of prostate-specific membrane antigen in the baculovirus expression system and recognition by prostate-specific membrane antigen-specific T-cells.*J Immunother* 22, 346-55 (1999)
35. Bosch, F. X., Manos, M. M., Munoz, N., Sherman, M., Jansen, A. M., Peto, J., Schiffman, M. H., Moreno, V., Kurman, R., and Shah, K. V. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group.*J Natl Cancer Inst* 87, 796-802 (1995)
36. Walboomers, J. M., Jacobs, M. V., Manos, M. M., Bosch, F. X., Kummer, J. A., Shah, K. V., Snijders, P. J., Peto, J., Meijer, C. J., and Munoz, N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide.*J Pathol* 189, 12-9 (1999)
37. Howley, P. M. Papillomavirinea: The viruses and their replication. In: Fields virology. Fields BN, Knipe DM, Howley PM, Chanock RM, Melnick JL, Monath TP, Roizman B, Straus SE (Eds.), Littincott-Raven, Philadelphia 2045-76 (1996)
38. Dyson, N., Howley, P. M., Munger, K., and Harlow, E. The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product.*Science* 243, 934-7 (1989)
39. Wang, F., Bade, E., Kuniyoshi, C., Spears, L., Jeffery, G., Marty, V., Groshen, K., and Weber, S. Phase I trial of a MART-1 peptide vaccine with incomplete Freund's adjuvant for resected high-risk melanoma.*Clin Cancer Res* 5, 2756- 65 (1999)
40. Frazer, I. Vaccines for papillomavirus infection.*Virus Res* 89, 271-4 (2002)
41. Feltkamp, M. C., Smits, H. L., Vierboom, M. P., Minnaar R. P., de Jongh, B. M., Drijfhout, J. W., ter Schegget, J., Melief, C. J., and Kast, W. M. Vaccination with cytotoxic T lymphocyte epitope-containing peptide protects against a tumor induced by human papillomavirus type 16-transformed cells.*Eur J Immunol* 23, 2242-9 (1993)
42. Steller, M. A., Gurski, K. J., Murakami, M., Daniel, R. W., Shah, K. V., Celis, E., Sette, A., Trimble, E. L., Park, R. C., and Marincola, F. M. Cell-mediated immunological responses in cervical and vaginal cancer patients immunized with a lipidated epitope of human papillomavirus type 16 E7.*Clin Cancer Res* 4, 2103-9 (1998)
43. van Driel, W. J., Ressing, M. E., Kenter, G. G., Brandt, R. M., Krul, R. J., van Rossum, A. B., Schuuring, E., Offringa, R., Bauknecht, T., Tamm-Hermelink, A., van Dam, P. A., Fleuren, G. J., Kast, W. M., Melief, C. J., and Trimbos, J. B. Vaccination with HPV16 peptides of patients with advanced cervical carcinoma: clinical evaluation of a phase I-II trial.*Eur J Cancer* 35, 946-52 (1999)
44. Ressing, M. E, van Driel, W. J., Brandt, R. M., Kenter, G. G., de Jong, J. H., Bauknecht, T., Fleuren, G. J., Hoogerhout, P., Offringa, R., Sette, A., Celis, E., Grey, H., Trimbos, B. J., Kast, W. M., and Melief, C. J. Detection of T helper responses, but not of human papilloma virus-specific cytotoxic T lymphocyte responses, after peptide vaccination of patients with cervical carcinoma.*J Immunother* 23, 255-66 (2000)
45. Muderspach, L., Wilczynski, S., Roman, L., Bade, L., Felix, J., Small, L. A., Kast, W. M., Fascio, G., Marty, V., and Weber, J. A phase I trial of a human papilloma virus (HPV) peptide vaccine for women with high-grade cervical and vulvar intraepithelial neoplasia who are HPV 16 positive.*Clin Cancer Res* 6, 3406-16 (2000)
46. Harper, D. M., Franco, E. L., Wheeler, C. M., Moscicki, A. B., Romanowski, B., Roteli-Martins, C. M., Jenkins, D., Schuind, A., Costa Clemens, S. A., and Dubin, G. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papilloma virus types 16 and 18: follow-up from a randomised control trial.*Lancet* 367, 1247-55 (2006)
47. Abrams, S. I., Khleif, S. N., Bergmann-Leitner, E. S., Kantor, J. A., Chung, Y., Hamilton, J. M., and Schlom, J. Generation of stable CD4+ and CD8+ T cell lines from patients immunized with ras oncogene-derived peptides reflecting codon 12 mutations.*Cell Immunol* 182, 137-51 (1997)
48. Khleif, S. N., Abrams, S. I., Hamilton, J. M., Bergmann-Leitner, E., Chen, A., Bastian, A., Bernstein, S., Chung, Y., Allegra, C. J., and Schlom, J. A phase I vaccine trial with peptides reflecting ras oncogene mutations of solid tumors.*J Immunother* 22, 155-65 (1999)
49. Carbone, D. M., Kelley, M. C., Smith, E. V., Maher, D., Kavanaugh, S., Nadaf, M., Stipanov, A., Contois, D., Ciernik, I. F., Johnson, B. E., Pendelton, C. D., Kelsey, M., Carter, C., Steindberg, S., Minna, J. D., and Berzofsky, J. A. Results of NCI T93-0148: detection of immunologic responses and vaccination against tumor specific mutant ras and p53 peptides in patients with cancer.*Proc ASCO* 18, 430-8 (1999)
50. Gjertsen, M. K., Buanes, T., Rosseland, A. R., Bakka, A., Gladhaug, I., Soreide, O., Eriksen, J. A., Moller, M., Baksaas, I., Lothe, R. A., Saeterdal, I., and Gaudernack, G. Intradermal ras peptide vaccination with granulocyte-macrophage colony-stimulating factor as adjuvant: Clinical and immunological responses in patients with pancreatic adenocarcinoma.*Int J Cancer* 92, 441-50 (2001)
51. Wang, R. F., and Rosenberg, S. A. Human tumor antigens for cancer vaccine d evelopment.*Immunol Rev* 170,185-200 (1999)
52. Rosenberg, S. A. Cancer vaccines based on the identification of genes encoding cancer regression antigens.*Immunol Today* 18, 175-82 (1997)
53. Kawakami, Y., Robbins, P. F., Wang, R. F., Parkhurst, M., Kang, X., and Rosenberg, S. A. The use of melanosomal proteins in the immunotherapy of melanoma.*J Immunother* 21, 237-46 (1998)

54. Weber, J. S., and Aparicio, A. Novel immunologic approaches to the management of malignant melanoma.*Curr Opin Oncol* 13, 124-8 (2001)
55. Jager, D., Jager, E., and Knuth, A. Vaccination for malignant melanoma: recent developments.*Oncology* 60, 1-7 (2001)
56. Wang, E., Phan, G. Q., and Marincola, F. M. T-cell-directed cancer vaccines: the melanoma model.*Expert Opin Biol Ther* 1, 277-90 (2001)
57. Davis, I. D., and Lotze, M. T. Melanoma peptide clinical trials. In: peptide-based cancer vaccines. Ed: W. M. Kast, Landes Bioscience, Georgetown, TX, 215- 36 (2000)
58. Reynolds, S. R., Oratz, R., Shapiro, R. L., Hao, P.,Yun, Z., Fotino, M., Vukmanović, S., and Bystryn, J. C. Stimulation of $CD8^+$ T cell responses to MAGE-3 and Melan A/MART-1 by immunization to a polyvalent melanoma vaccine.*Intl J Cancer* 72, 972-6 (1998)
59. Talwar, G. P., Gupta, S. K., Singh, V., Sahal, D., Iyer, K. S., and Singh, O. Bioeffective monoclonal antibody against the decapeptide gonadotropin-releasing hormone: reacting determinant and action on ovulation and estrus suppression.*Proc Natl Acad Sci U S A* 82, 1228-31 (1985)
60. Ladd, A., Prabhu, G., Tsong, Y. Y., Probst, T., Chung, W., and Thau, R. B. Active immunization against gonadotropin-releasing hormone combined with androgen supplementation is a promising antifertility vaccine for males.*Am J Reprod Immunol Microbiol* 17, 121-7 (1988)
61. Thompson, D. L. Immunization against GnRH in male species (comparative aspects).*Anim Reprod Sci* 2, 459-69 (2000)
62. Miller, L. A., Johns, B. E., and Killian, G. J. Immunocontraception of white- tailed deer with GnRH vaccine.*Am J Reprod Immunol* 44, 266-74 (2000)
63. Kiyama, Z., Adams, T. E., Hess, B. W., Riley, M. L., Murdoch, W. J., and Moss, G. E. Gonadal function, sexual behavior, feedlot performance and carcass traits of ram lambs actively immunized against GnRH.*J Anim Sci* 78, 2237-43 (2000)
64. Jayashankar, R., Chaudhuri, M. K., Singh, O., Alam, A., and Talwar, G. P. Semisynthetic anti-LHRH vaccine causing atrophy of the prostate.*Prostate* 14, 3-11 (1989)
65. Ferro, V. A., and Stimson, W. H. Antigonadotropin releasing hormone vaccines and their potential use in the treatment of the hormone responsive cancers.*BioDrugs* 12, 1-12 (1999)
66. Simms, M. S., Scholfield, D. P., Jacobs, E., Michaeli, D., Broome, P., Humphreys, J. E., and Bishop, M. C. Anti-GnRH antibodies can induce castrate levels of testosterone in patients with advanced prostate cancer.*Br J Cancer* 83, 443-6 (2000)
67. Talwar, G. P., Hingorani, V., Kumar, S., Roy, S., Banerjee, A., Shahani, S. M., Krishna, U., Dhall, K., Sawhney, H., and Sharma, N. C. Phase I clinical trials with three formulations of anti-human chorionic gonadotropic vaccine.*Contraception* 41, 301-16 (1990)
68. Jones, W. R., Bradley, J., Judd, S. J., Denholm, E. H., Ing, R. M., Mueller, U. W., Powell, J., Griffin, P. D., and Stevens, V. C. Phase I clinical of a World Health Organization birth control vaccine.*Lancet* I, 1295-8 (1998)
69. Talwar, G. P., Singh, O., Pal, R., Chatterjee, N., Sahai, P., Dhall, K., Kaur, J., Das, S. K., Suri, S., Buckshee, K., Saraya, L., and Saxena, B. N. A vaccine that prevents pregnancy in women.*Proc Natl Acad Sci U S A* 91, 8532-6 (1994)
70. Yanagimachi, R. Mammalian fertilization. In: Knobil E. Neill J, editors. The Physiology of Reproduction. New York: Raven Press, 138-85 (1988)
71. Wassarman, P. M. Mammalian fertilization: molecular aspects of gamete adhesion, exocytosis, and fusion.*Cell* 96, 175-83 (1999)
72. Rankin, T., Familari, M., Lee, E., Ginsberg, A., Dwyer, N., Blanchette-Mackie, J., Drago, J., Westphal, H., and Dean, J. Mice homozygous for an insertional mutation in the Zp3 gene lack a zona pellucida and are infertile.*Development* 122, 2903-10 (1996)
73. Naz, R. K., Sacco, A., Singh, O., Pal, R., and Talwar, G. P. Development of contraceptive vaccines for humans using antigens derived from gametes (spermatozoa and zona pellucida) and hormones (human chorionic gonadotrophin): current status.*Hum Reprod Update* 1, 1-18 (1995)
74. Aitken, J. R. Immunocontraceptive vaccines for human use.*J Reprod immunol* 57, 273-87 (2002)
75. Sacco, A. G., Subramanian, M. G., Yurewicz, E. C., DeMayo, F. J., and Dukelow, W. R. Heteroimmunization of squirrel monkeys (Saimiri sciureus) with a purified porcine zona antigen (PPZA): immune response and biologic activity of antiserum.*Fertil Steril* 39, 350-8 (1983)
76. Dunbar, B. S, Lo, C., Powell, J., and Stevens, V. C. Use of a synthetic peptide adjuvant for the immunization of baboons with denatured and deglycosylated pig zona pellucida glycoproteins.*Fertil Steril* 52, 11-8 (1989)
77. Keenan, J. A., Sacco, A. G., Subramanian, M. G., Kruger, M., Yurewicz, E. C., and Moghissi, K. S. Endocrine response in rabbits immunized with native versus deglycosylated porcine zona pellucida antigens. *Biol Reprod* 44, 150-6 (1991)
78. Millar, S. E, Chamow, S. M., Baur, A. W., Oliver, C., Robey, F., and Dean, J. Vaccination with a synthetic zona pellucida peptide produces long-term contraception in female mice.*Science* 246, 935-8 (1989)
79. Govind, C. K., and Gupta, S. K. Failure of female baboons (*Papio anubis*) to conceive following immunization with recombinant non-human primate zona pellucida glycoprotein-B expressed in Escherichia coli.*Vaccine* 18, 2970-8 (2000)
80. Sun, W., Lou, Y. H., Dean, J., and Tung, K. S. A contraceptive peptide vaccine targeting sulfated glycoprotein ZP2 of the mouse zona pellucida.*Biol Reprod* 60, 900-7 (1999)
81. Hasegawa, A., Hamada, Y., Shigeta, M., and Koyama, K. Contraceptive potential of synthetic peptides of zona pellucida protein (ZPA).*J Reprod Immunol* 53, 91- 8 (2002)
82. Sivapurapu, N., Upadhyay, A., Hasegawa, A., Koyama, K., and Gupta, S. K. Native zona pellucida reactivity and in-vitro effect on human sperm-egg binding with antisera against bonnet monkey ZP1 and ZP3 synthetic peptides.*J Reprod Immunol* 56, 77-91 (2002)
83. Ringleb, J., Rohleder, M., and Jewgenow, K. Impact of feline zona pellucida glycoprotein B-derived synthetic peptides on in vitro fertilization of cat oocytes.*Reproduction* 127, 179-86 (2004)
84. Lou, Y., Ang, J., Thai, H., McElveen, F., and Tung, K. S. A zona pellucida 3peptide vaccine induces antibodies

and reversible infertility without ovarian pathology.*J Immunol* 155, 2715-20 (1995)
85. Paterson, M., Wilson, M. R., Jennings, Z. A., van Duin, M., and Aitken, R. J. Design and evaluation of a ZP3 peptide vaccine in a homologous primate model.*Mol Hum Reprod* 5, 342-52 (1999)
86. Kaul, R., Sivapurapu, N., Afzalpurkar, A., Srikanth, V., Govind, C. K., and Gupta, S. K. Immunocontraceptive potential of recombinant bonnet monkey (Macaca radiata) zona pellucida glycoprotein-C expressed in Escherichia coli and its corresponding synthetic peptide.*Reprod Biomed Online* 2, 33-9 (2001)
87. Miller, L. A., and Killian, G. J. In search of the active PZP epitope in white-tailed deer immunocontraception.*Vaccine* 20, 2735-42 (2002)
88. Hardy, C. M., ten Have, J. F., Mobbs, K. J., and Hinds, L. A. Assessment of the immunocontraceptive effect of a zona pellucida 3 peptide antigen in wild mice.*Reprod Fertil Dev* 14,151-5 (2002)
89. Bagavant, H., Francesco, M. F., Baischi, J., Kurth, B., and David, C. S. Immunogenicity and contraceptive potential of a human zona pellucida 3 peptide vaccine.*Biol Reprod* 56, 764-77 (1997)
90. Srivastava, N., Santhanam, R., Sheela, P., Mukund, S., Thakral, S. S., Malik, B. S., and Gupta, S. K. Evaluation of the immunocontraceptive potential of Escherichia coli-expressed recombinant dog ZP2 and ZP3 in a homologous animal model.*Reproduction* 123, 847-57 (2002)
91. Miller, L. A., and Killian, G. J. In search of the active PZP epitope in white-tailed deer immunocontraception.*Vaccine* 20, 2735-42 (2002)
92. Fayrer-Hosken, R. A., Grobler, D., van Altena, J. J., Bertschinger, H. J., and Kirkpatrick, J. F. Immunocontraception of African elephants.*Nature* 407, 149- 50 (2000)
93. Naz, R. K., Alexander, N. J., Isahakia, M., and Hamiltion, M. D. Monoclonal antibodies to a human sperm membrane glycoprotein that inhibit fertilization.*Science* 225, 342-4 (1984)
94. Naz, R. K., Ahmad, K., and Kumar, R. Role of membrane phosphotyrosine proteins in human spermatozoal function.*J Cell Sci* 99, 157-65 (1991)
95. Zhu, X., and Naz, R. K. Fertilization antigen-1: cDNA cloning, testis-specific expression, and immunocontraceptive effects.*Proc Natl Acad Sci USA* 94, 4704- 9 (1997)
96. Naz, R. K., and Zhu, X. Molecular cloning and sequencing of cDNA encoding for human FA-1 antigen.*Mol Reprod Devel* 63, 256-68 (2002)
97. Naz, R. K., and Zhu, X. Recombinant fertilization antigen-1 causes a contraceptive effect in actively immunized mice.*Biol Reprod* 59, 1095-100 (1998)
98. Naz, R. K., Zhu, X., and Kadam, A. L. Identification of human sperm peptide sequence involved in egg binding for immunocontraception.*Biol Reprod* 62, 318- 24 (2000)
99. Naz, R. K., and Chauhan, S. C. Presence of antibodies to sperm YLP_{12} synthetic peptide in sera and seminal plasma of immunoinfertile men.*Mol Hum Reprod* 7, 21-6 (2001)
100. Naz, R. K., and Chauhan, S. C. Human sperm-specific peptide vaccine that causes long-term reversible contraception.*Biol Reprod* 64, 674-80 (2002)
101. O'Rand, M. G., Widgren, E. E., and Fisher, S. J. Characterization of the rabbit sperm membrane autoantigen, RSA, as a lectin-like zona binding protein. *Dev Biol* 129, 231-40 (1988)
102. O'Rand, M. G., Beavers, J., Widgren, E., and Tung, K. Inhibition of fertility in female mice by immunization with a B-cell epitope, the synthetic sperm peptide, P10G.*J Reprod Immunol* 25, 89-102 (1993)
103. Richardson, R. T., Yamasaki, N., and O'Rand, M.G. Sequence of a rabbit sperm zona pellucida binding protein and localization during the acrosome reaction.*Dev Biol* 165, 688-701 (1994)
104. Lea, I. A., van Lierop, M. J. C., Widgren, E. E., Grootenhuis, A., Wen, Y., van Duin, M., and O'Rand, M. G. A chimeric sperm peptide induces antibodies and strain-specific reversible in fertility in mice.*Biol Reprod* 59, 527-36 (1998)
105. Brookbinder, L. H., Cheng, A., and Bleil, J. D. Tissue- and species-specific expression of sp56, a mouse sperm fertilization protein.*Science* 269, 86-9 (1995)
106. Hardy, C. M., and Mobbs, K. J. Expression of recombinant mouse sperm protein sp56 and assessment of its potential for use as an antigen in an immunocontraceptive vaccine.*Mol Reprod Develop* 52, 527-36 (1999)
107. Hardy, C. M., Clydesdale, G., and Mobbs, K. J. Development of mouse-specific contraceptive vaccines: infertility in mice immunized with peptide and polyepitope antigens.*Reprod* 128, 395-404 (2004)
108. Haneji, T., and Koide, S. S. Identification of antigen in rat spermatogenic cellsinteracting with an anti-human sperm monoclonal antibody.*Biol Reprod* 37, 467-77 (1987)
109. Vange, G., Lu, Y. A., Tam, J. P., and Koide, S. S. Infertility induced in rats by immunization with synthetic peptide segments of a sperm protein.*B B R C* 183, 538-43 (1992)
110. Bai, Y., Miao, S. Y., Yan, Y. C., Wang, L. F., and Koide, S. S. Nucleic acid sequence of cDNA (RSD-1) encoding a rabbit sperm membrane protein and structural properties of the encoded protein.*Proc Chinese Acda Med Sci and Peking Union Med Coll* 5, 181-5 (1990)
111. Vange, G. R., Jaiswal, Y. K., Lu, Y. A., Tam, J. P., Wang, L. F., and Koide, S. S. Immunization with synthetic peptide segments of a sperm protein impair fertility in rats.*Res Commun Chem Pathol Pharmacol* 84, 3-15 (1994)
112. Goldberg, E., and Herr, J. C. LDH-C4 as a contraceptive vaccine. In: Gupta, S. K., editor. Reproductive Immunology. New Delhi, India: Narosa Publishing House, 309-15 (1999)
113. O'Hern, P. A., Liang, Z. G., Bambra, C. S., and Goldberg, E. Colinear synthesis of an antigen-specific B-cell epitope with a "promiscuous" tetanus toxin T-cell epitope: a synthetic peptide immunocontraceptive.*Vaccine* 35, 1761-6 (1997)
114. Toller, T. L., Overstreet, J. W., Branciforte D., and Primakoff, P. D. Immunization of female Cynomolgus macaques with a synthetic epitope of a sperm-specific lactate dehydrogenase results in high antibody titres but does not reduce fertility.*Mol Reprod Develop* 62, 257-64 (2002)
115. Naz, R. K., and Aleem, A. Effect of immunization with six sperm peptide vaccines on fertility of female mice.*Reproduction,* in press (2006)
116. Berzoksky, J. A., Ahlers, J. D., and Belyakov, I. M. Strategies for designing andoptimizing new generation vaccines.*Nature* 1, 209-19 (2001)

117. Pardoll, D. M. Spinning molecular immunology into successful immunotherapy.*Nature* 2, 227-38 (2002)

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