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MUC1 and Le^Y are cell surface antigens expressed on a variety of epithelial cancers including cancers of the breast and ovary. They would appear to be excellent targets for antibody inducing vaccines. MUC1 and Le^Y vaccines prepared and tested over the last 4 years have resulted in high titer antibodies against the synthetic antigens which were of relatively modest titer against tumor cells expressing these antigens. While these vaccines could be included in future polyvalent vaccines, it was our impression that we could augment the relevant immunogenicity by using longer MUC1 peptides or glycosylated peptides, or by using Le^Y vaccines with a higher Le^Y/ KLH epitope ratio. Consequently, a series of second generation MUC1 and Le^Y vaccines have been prepared, tested for safety and immunogenicity in mice and are now in clinical trials. Trials with the first two, a longer MUC1 peptide (106aa) and a glycosylated version of this longer MUC1 peptide, have been completed and the serologic results are not better than with the original shorter, unglycosylated MUC1. Results of the currently ongoing trials with shorter glycosylated MUC1 peptides and with the higher epitope ratio Le^Y are not yet available.

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INTRODUCTION

Due to the 75% reduction in funding level from our original grant application the work scope has been restricted to the production and pre-clinical testing of MUC1 and Lewis Y vaccines for patients with breast cancer or ovarian cancer. The goal of the trials is to induce antibodies against MUC1 and Lewis Y which are cell surface antigens broadly expressed on cancers of the ovary and breast. Clinical trials with both preparations have been conducted over the last 3 years and results are available. Several modified versions (second generation) of these two vaccines have been prepared and tested or are being tested in the clinic.

BODY

MUC1

Objective: To select a MUC1 peptide in a MUC1-KLH (keyhole limpet hemocyanin) conjugate vaccine that generates the optimal immune response against MUC1 peptide and tumor cells expressing MUC1.

Methods: We had previously immunized breast cancer patients with a MUC1-KLH (Keyhole Limpet Hemocyanin) plus QS-21 adjuvant vaccine containing 1 ½ repeats of the MUC1 20 amino acid (aa) tandem repeat (1). This 32aa MUC1 vaccine induced high titer antibodies against MUC1 in essentially all immunized patients but these antibodies reacted only moderately with the cell surface of tumor cells expressing MUC1 (2). Consequently, a variety of modifications of the MUC1 peptide have been synthesized, prepared as KLH conjugates and tested. These were either longer versions of the MUC1 peptide or glycosylated MUC1, in both cases the goal was to make the MUC1 more closely approximate the way MUC1 appears on the tumor cell surface. The results of the completed trials are summarized in Table 1.

A 106aa MUC1 peptide expressing more than 5 repeats of the 20aa MUC1 tandem repeat was prepared. This was no simple feat. This long peptide was prepared with a terminal cystine for linkage to KLH. Since the conjugation efficiency is only 15%, 30mg of the MUC1 peptide were required. The peptide was purified to exclude shorter MUC1 peptides, sequenced to confirm the proper sequence and conjugated to KLH using an M-maleimidobenzoyl-N-hydroxy succinimide (MBS) as previously described (1). Unbound MUC1 was excluded with a 30,000 molecular weight filter and the conjugate mixed with QS-21 and vialed. The epitope ratio of MUC1 to KLH was 560 to 1. Vials were opened to confirm sterility, purity, safety and immunogenicity as required by the FDA.

MUC1 peptides (106aa or 30-32aa) were glycosylated with 0, 1, 3 or 5 Tn epitopes (N-acetylgalactosamine) per 20 amino acid tandem repeat (TR), as indicated in the Table below, and processed as described above. Small groups of patients with treated breast cancer or ovarian cancer and no evidence of current disease were immunized subcutaneously with one of these various MUC1-KLH conjugate preparations plus immunological adjuvant QS-21 on weeks 0, 1, 2, 6 and 18. Sera were analyzed for ELISA reactivity against the immunizing MUC1 peptides and by FACS against the

MUC1 positive breast cancer cell line MCF7. T-cell proliferation and IFN-γ-release ELISPOT assays to the immunizing peptide was measured by tritiated thymidine incorporation after a 5 day invitro sensitization

Results: All vaccines were well tolerated with the only toxicity being local erythema and induration at vaccination sites lasting 2-5 days and occasional mild flu-like symptoms lasting 1-2 days. Serologic responses are summarized below. While potent T-cell proliferation and ELISPOT reaction with KLH were seen, no consistent evidence of proliferation or ELISPOT reactivity against MUC1 was identified.

TABLE 1

Vaccine (+QS-21)	Glycosylation No. of sites per TR	No. of Amino Acids	No. of Patients Vaccinated	Median Serological Reactivity* ELISA		FACS(MCF7) % Pos Cells	
				IgM	IgG	IgM	IgG
KLH-MUC1(-APDTRPA)†	0	31	9	1280	10240	52%	26%
KLH-MUC1(-RPAPGST)	0	33	10	1280	320	35%	8%
(HGVTSAP-) MUC1	0	106	11	10	0	14%	13%
(HGVTSAP-) MUC1-KLH	0	106	13	1280	1280	29%	14%
KLH-MUC1(-AHGVTSA)	1	31	-	Accrual ongoing			
KLH-MUC1(-APDTRPA)	3	33	-	Accrual completed			
KLH-MUC1(-APDTRPA)	5	33	-	Accrual ongoing			
(HGVTSAP-)MUC1-KLH	5	106	18	80	40		

**Median pretreatment ELISA titers 0, median pretreatment percent positive cells 10-11%*

† Sequence in parenthesis indicates N- or C- terminal sequence of peptide away from KLH.

Lewis Y (Le^Y)

Lewis Y pentasaccharide was synthesized as the allyl glycoside as described previously. It was conjugated to KLH following reductive amination with an Le^Y-KLH conjugate ratio of 310/1. The yield of conjugated Le^Y in this reaction was 8%. Le^Y-KLH conjugate was vialed at four different concentrations with QS-21 and the vials tested for sterility, safety, and immunogenicity. Twenty-four patients were vaccinated with vaccines containing 3, 10, 30 or 60mg of Le^Y in-groups of six patients (3). The peak titer IgM and IgG ELISA results against Le^Y

and the pre and post immunization flow cytometry results at the four different vaccine doses are demonstrated in the table below. The 10μg dose was selected for testing in future vaccination trials. However, the ELISA titers and flow cytometry results were not as striking as initially hoped and so second generation Le^Y vaccines were prepared. The first was the same except that an improved Le^Y to KLH ratio (600/1) was achieved. A trial with this vaccine was recently initiated in patients with ovarian cancer. An additional vaccine containing Le^Y clusters has been prepared and is currently being tested in mice. This contains three Le^Y pentasaccharides linked to alternating serines on a short peptide chain with a terminal cystine, which is used for linkage to KLH.

TABLE 2

Summary of Serological Responses to Vaccination with Le^Y-KLH+QS21

Vaccine Le^Y Dose	No of Patients	Peak Median ELISA Titer IgM	IgG	Median Peak FACS % Positive Cells	Median CDC % Lysis
3μg	6	20	0	10	7.3
10μg	6	80	0	26	29
30μg	6	40	0	24	19
60μg	6	20	0	8.6	7

KEY RESEARCH ACCOMPLISHMENTS

1) Preparation of a 106aa MUC1 peptide with proper sequence for conjugation to KLH and vaccine production.

2) Preparation of a series of MUC1-KLH vaccines and completion of pre-clinical and clinical testing.

3) Synthesis of Le^Y pentasaccarides for vaccine production.

4) Preparation of Le^Y conjugate vaccines and completion of pre-clinical and clinical testing.

5) Preparation of second generation MUC1 and Le^Y vaccines containing glycosylated MUC1, higher epitope ratio Le^Y, and Le^Y clusters.

REPORTABLE OUTCOMES

Pending results of currently ongoing trials

CONCLUSIONS

The MUC1 and Le^Y vaccines prepared and tested over the last year have resulted in high titer antibodies against the synthetic antigens which were of relatively modest titer against tumor cells expressing these antigens. While these vaccines could be included in future polyvalent vaccines, it is our impression that we can augment the relevant immunogenicity by the modifications demonstrated. Consequently, a series of second generation MUC1 and Le^Y vaccines have been prepared, tested for safety and immunogenicity in mice and are now in clinical trials. Trials with the first two, a longer MUC1 peptide (106aa) and a glycosylated version of this longer MUC1 peptide, have been completed and the serologic results are not better than with the original shorter, unglycosylated MUC1. Results of the currently ongoing trials with shorter glycosylated MUC1 peptides and with the higher epitope ratio Le^Y are not yet available.

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