Vaccination of CEA-transgenic mice with a recombinant *Saccharomyces cerevisiae*-CEA vaccine breaks immune tolerance and elicits therapeutic antitumor responses

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Abstract

Studies were designed to determine if vaccination of human carcinoembryonic antigen (CEA)-transgenic mice (where CEA is a self-antigen) with a heat-killed recombinant *S. cerevisiae* construct expressing human CEA (yeast-CEA) elicits dendritic cell (DC) activation, CEA-specific T-cell responses and antitumor activity. CEA-transgenic mice were vaccinated with yeast-CEA, and maturation of DCs was studied. In addition, CD4+ and CD8+ T-cell responses were assessed after one and multiple administrations or vaccinations at multiple sites per administration. Antitumor activity was determined by tumor growth and overall survival in both pulmonary metastasis and subcutaneous pancreatic tumor models. These studies demonstrate that recombinant yeast can indeed break tolerance and that a) vaccination with yeast-CEA induces the maturation of DCs; b) treatment of DCs with yeast-CEA resulted in specific activation of CEA-specific CD8+ T cells *in vitro* in an MHC-restricted manner; c) yeast-CEA constructs can elicit both CEA-specific CD4+ and CD8+ T-cell responses *in vivo*; d) repeated yeast-CEA administration causes increased antigen-specific T-cell responses after each vaccination; e) vaccination with yeast-CEA at multiple sites induces a greater T-cell response than the same dose given at a single site; f) tumor-bearing mice vaccinated with yeast-CEA show a reduction in tumor burden and increased overall survival compared to mock-treated or control yeast-vaccinated mice in both pulmonary metastasis and subcutaneous pancreatic tumor models. These studies thus form the rationale for the incorporation of recombinant yeast-CEA and other recombinant yeast constructs in cancer immunotherapy protocols.

Figure 1.

Yeast Immunogen Platform

Heat-killed Recombinant *S. cerevisiae* transfected with gene for target antigen has been used as therapeutic vaccine in different preclinical studies (Cancer and Infectious disease settings)

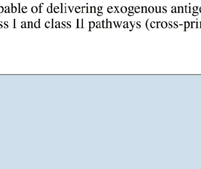

Figure 2.

Yeast-Based Vaccines: Mechanism of Action

Injected heat-killed recombinant yeast binds to mannose receptors on surface of dendritic cells

↓

Yeast are phagocytosed and internalized by DC (4hr)

↓

Recombinant protein released in phagolysosome and gains access to proteasomes in the cytosol

↓

Antigen is processed and presented on MHC I and MHC II receptors

↓

Effective stimulation of CD4 and CD8 T-cells

*Yeast can mature and stimulate DC independently of TLR signaling

Figure 3.

Advantages of Recombinant Yeast as a Vaccine Vector

- Safe, heat-killed, no biosafety requirements
- Easily engineered to express antigen(s)
- Cultured rapidly in large quantities
- Stable, easily transported
- Non-toxic
- Immunostimulatory complex that may abrogate need for additional adjuvants
- Capable of delivering exogenous antigen to both class I and class II pathways (cross-priming)

Figure 4.

Exposure of Immature Murine Dendritic Cells to Recombinant Yeast-CEA Results in Phenotypic Maturation

DC+Yeast-CEA → 48h → FACS, Cytokine

Phenotype — DC Panel [% positive cells (MFI)]

DC Treatment	I-A^b	H-2K^b/D^b	CD11b	CD11c	CD40	B7-2	B7-1	ICAM-1	LFA-3	CD45
Media	75 (1109)	96 (923)	24 (158)	31 (209)	47 (259)	50 (794)	60 (455)	92 (870)	84 (482)	31 (105)
LPS	**90 (616)**	97 (205)	27 (43)	**34 (88)**	**67 (133)**	**61 (694)**	**79 (202)**	**95 (1292)**	**86 (366)**	**73 (68)**
Yeast-CEA	**89 (1846)**	**97 (1274)**	22 (160)	**50 (383)**	**68 (387)**	**70 (1103)**	**83 (708)**	**99 (1124)**	**95 (653)**	33 (135)

Cytokine — Cytokine (pg/mL)

DC Treatment	IL-12	TNF-α	IFN-γ	IL-10	IL-6	IL-5	IL-4	IL-2	MCP-1
Media	41	60	20	50	80	<4	<4	<4	14,200
Yeast-CEA	**75**	**5,020**	**102**	**225**	**1,869**	<4	<4	<4	**16,454**

Figure 5.
Exposure of immature dendritic cells to recombinant Yeast-CEA results in maturation. DC were derived from C57B6 mouse bone marrow treated with GM-CSF and IL-4. On day 5, DC were incubated with Yeast-CEA at a ratio of 1:1. As a control, DC were matured with LPS (50 μg/ml). After 48h, cell were analyzed by flow cytometry. Supernatant fluids were analyzed by cytometric bead array. Upper panel: Bold numbers indicate a significant change in either % positive cells or cell-surface expression (MFI) as compared with no treatment. Lower Panel: Bold numbers indicate a significant change in as compared with no treatment as determined by the Kolmogorov-Smirnov test.

DC Treated with Yeast-CEA Stimulate CEA-Specific T-cells

DC+Yeast-CEA (1:1) → 48h → CEA T-cell Line (Ratio 4:1) → 24h → IFN-gamma

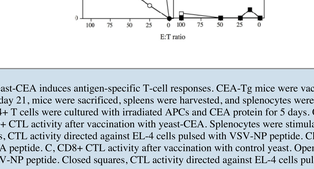

Figure 6.
Dendritic cells treated with Yeast-CEA stimulate CEA-Specific T-cells.
DC were derived from C57B6 mouse bone marrow treated with GM-CSF and IL-4. On day 5, DC were incubated with either Yeast-CEA or Yeast-Control at a ratio of 1:1 for 48 hours. DC were then co-cultured with CEA specific T-cells at a ratio of 4:1 for 24 hours. Supernatant fluids were analyzed for IFN-γ after 2 days by cytometric bead array. For MHC blocking studies, anti-H2Db mAb or isotype control antibody was added at 10 μg/ml for the duration of the experiment.

Vaccination with Yeast-CEA Induces CEA-specific T-cell Proliferation

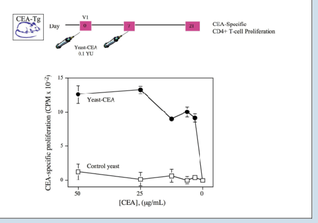

Figure 7.
See Legend Below (Figure 7 and 8)

Vaccination with Yeast-CEA Induces CEA-specific CTL Activity

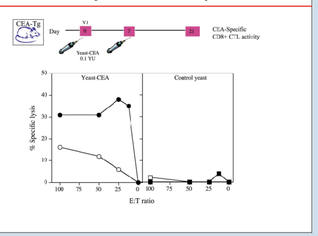

Figure 7 and 8.
Vaccination with yeast-CEA induces antigen-specific T-cell responses. CEA-Tg mice were vaccinated with 0.1 YU control yeast or yeast-CEA on days 0 and 7. On day 21, mice were sacrificed, spleens were harvested, and splenocytes were used for assays. Figure 7: CD4+ cell proliferation. Purified CD4+ T cells were cultured with irradiated APCs and CEA protein for 5 days. Open squares, control yeast. Closed circles, yeast-CEA. Figure 8: CD8+ CTL activity after vaccination with yeast-CEA. Splenocytes were stimulated with CEA(572-579) peptide for 6 days before assays. Open circles, CTL activity directed against EL-4 cells pulsed with VSV-NP peptide. Closed circles, CTL activity directed against EL-4 cells pulsed with CEA peptide. C, CD8+ CTL activity after vaccination with control yeast. Open squares, CTL activity directed against EL-4 cells pulsed with VSV-NP peptide. Closed squares, CTL activity directed against EL-4 cells pulsed with CEA peptide.

Efficacy of Multiple Yeast-CEA Vaccinations: CEA-Specific CD4+ T-cell Proliferation

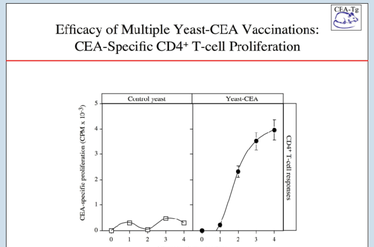

Figure 9.
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Efficacy of Multiple Yeast-CEA Vaccinations: CEA-Specific CD8+ T-cell Cytotoxicity

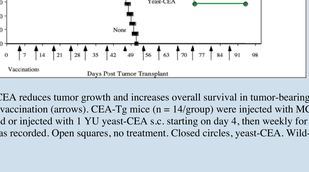


Figure 9 and 10.
Multiple vaccinations with yeast-CEA continuously increase T-cell responses. CEA-Tg mice were vaccinated with 0.1 YU control yeast or yeast-CEA 1, 2, 3, or 4 times at 7-day intervals. Fourteen days after the last vaccination, mice were sacrificed, spleens were harvested, and splenocytes were used for assays. Figure 9: CD4+ T-cell proliferation after vaccination with control yeast. Purified CD4+ T cells were cultured with irradiated APCs and CEA protein for 5 days. Figure 10: CD8+ CTL activity after vaccination with control yeast or yeast-CEA. Data are presented as % lysis after subtraction of VSV-NP control.

Optimal biologic dose of Yeast-CEA

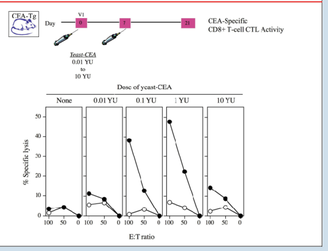


Figure 11.
CD8+ CTL responses after dose escalation of yeast-CEA. CEA-Tg mice were vaccinated with 0, 0.01, 0.1, 1, or 10 YU yeast-CEA twice at 7-day intervals. Fourteen days after the last vaccination, mice were sacrificed, spleens were harvested, and splenocytes were stimulated with CEA peptide for 6 days. Open circles, EL-4 cells pulsed with VSV-NP peptide. Closed circles, EL-4 cells pulsed with CEA peptide.

Does Yeast-CEA have Antitumor Activity in a CEA Lung-Tumor Model?

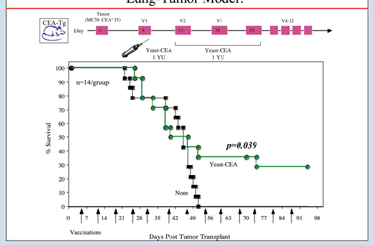
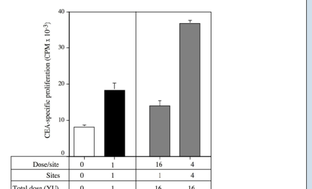


Figure 12.
Vaccination with yeast-CEA reduces tumor growth and increases overall survival in tumor-bearing mice. Survival in a lung metastasis model with continuous weekly vaccination (arrows). CEA-Tg mice (n = 14/group) were injected with MC38-CEA+ tumor cells i.v. in the tail on day 0, and were mock-treated or injected with 1 YU yeast-CEA s.c. starting on day 4, then weekly for the duration of the experiment. Mice were monitored and survival was recorded. Open squares, no treatment. Closed circles, yeast-CEA. Wild-Type Yeast had no antitumor effects (not shown).

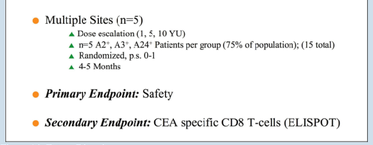
Yeast-CEA Injected into Multiple Sites vs. a Single site: CEA Specific CD4+ T-cell Proliferation



Dose/site	0	1	16	4
Sites	0	1	1	4
Total dose (YU)	0	1	16	16

Figure 13.
CD4+ T-cell responses increase when vaccine is distributed to multiple sites. CEA-Tg mice were vaccinated with a total of 1 or 16 YU yeast-CEA s.c. in 1 or 4 sites on days 0 and 7. Fourteen days later, mice were sacrificed, spleens were harvested, and CD4+ T cells were purified. Cells were cultured with irradiated APCs and CEA protein for 5 days. Open bar, no treatment. Black bar, 1 YU in 1 site. Gray bars, 16 total YU.

Vaccination in Multiple Sites Increases Antitumor Efficacy



Dose/site	0	1	1	1	1
Sites	0	1	2	4	6
Total dose (YU)	0	1	2	4	6

Figure 14.
Vaccination in multiple sites increases antitumor efficacy. CEA-Tg mice were implanted with Panc02.CEA cells s.c. on day 0 and vaccinated in 0, 1, 2, 4, or 6 sites with 1 YU yeast-CEA/site starting on day 7, then weekly for the duration of the experiment. Tumor volume was measured twice a week and recorded. Groups: no treatment (n = 10), 1YU in 1 site (n = 9), 1 YU in 2 sites (n = 10), 1 YU in 4 sites (n = 10), 1 YU in 6 sites (n = 10). Bars indicate average tumor volume with SD. Open squares, no treatment. Closed circles, yeast-CEA.

Conclusions

- Yeast-CEA capable of stimulating and inducing maturation of DC
  - Up-regulation of co-stimulatory molecules
  - Increased production of inflammatory cytokines
- DC treated with yeast-CEA enhance allogeneic T-cell proliferation and effectively stimulate CEA-specific CD8 T-cells in a class I-dependent manner
- Vaccination with Yeast-CEA induces CD4 and CD8 T cell responses
- Can be given multiple times with an increase in T cell responses after each vaccination
- Extends survival in mice in a CEA-positive lung metastasis cancer model
- Vaccinating in multiple sites is more effective than vaccinating in one site

Figure 15.

Phase I Trial: Dose Escalation of Sacchromyces-CEA (with 6D agonist epitope) Vaccine in Patients with CEA+ Carcinomas

- ***Classic Dose Escalation***
- ***Escalation of Number of Sites of Vaccination***
- Multiple Sites (n=5)
  - Dose escalation (1, 5, 10 YU)
  - n=5 $A2^+$, $A3^+$, $A24^+$ Patients per group (75% of population); (15 total)
  - Randomized, p.s. 0-1
  - 4-5 Months
- ***Primary Endpoint:*** Safety
- ***Secondary Endpoint:*** CEA specific CD8 T-cells (ELISPOT)

Figure 16. Future Directions