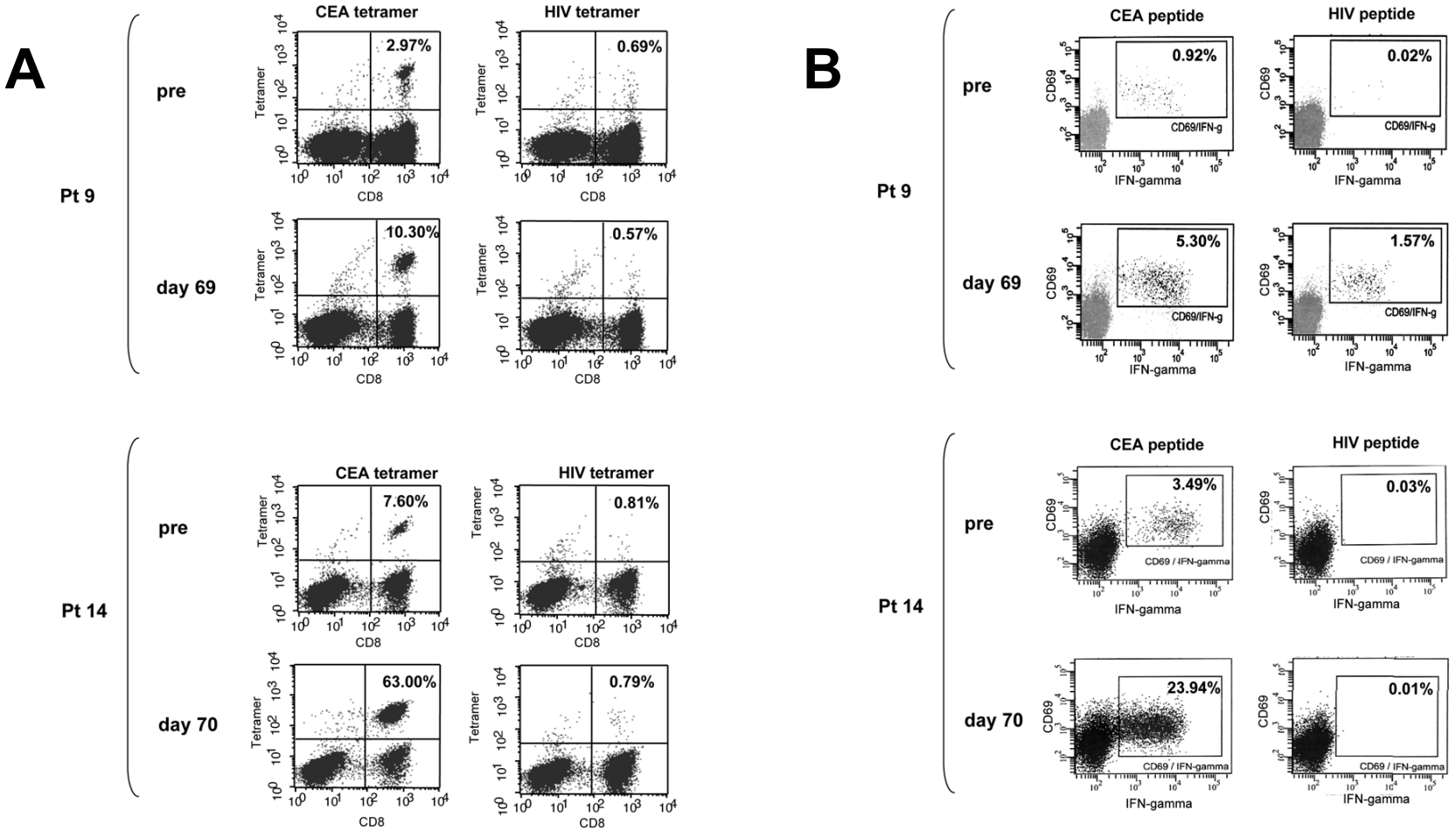
Supplementary Figure S1



Supplementary Figure S1 *A*, Flow cytometric analysis of CEA-MHC-tetramer binding for selected patients. Identification of CEA-specific CD8+ T cells in patients pre and post vaccination (day 69 and 70 as indicated. PE-labeled CEA-HLA-A*0201 tetramer or control tetramer (PE-labeled HIV-HLA-A*0201 tetramer) were used. T cells were stained with 10 ul of tetramers and anti-CD8 antibody for 30 min at room temperature (see Patients and Methods). Samples were analyzed in a FACScan and the CELLQuest program (BD Biosciences). Data gathered from 100,000 cells were stored and used to generate results.
B, Intracellular staining of IFN-gamma production by CEA-specific T cells. CEA-specific T cells (IVS 2) generated from blood from patients 9 and 14 both pre and post-vaccination (day 69 and day 70, as indicated) were *in vitro* stimulated with a CEA or an HIV-derived peptide and subsequently analyzed for intracellular IFN-gamma staining as described in Patients and Methods. Results are expressed as percentage of CD3+/CD8+/CD69+ T cells that are IFN-gamma positive.

Supplementary Table S1. Toxicities

Body system	Toxicity	Grade 2*	Grade 3*
Dermatologic	Injection-site reaction	88 (49%)	0 (0%)
Constitutional	Fatigue	5 (2.8%)	0 (0%)
	Fever	3 (1.7%)	0 (0%)
Blood/bone marrow	Hemoglobin	1 (<1%)	0 (0%)
Metabolic/laboratory	Hypoalbuminemia	1 (<1%)	0 (0%)
	Alk phos	1 (<1%)	0 (0%)
Neurological	Syncope	0 (0%)	2 (1.1%)**
Gastrointestinal	Anorexia	1 (<1%)	0 (0%)
	Vomiting	1 (<1%)	0 (0%)
Endocrine	Hot flushes/flashes	1 (<1%)	0 (0%)

*Number (%) of vaccines associated with event possibly, likely, or definitely related to vaccine. Total of 180 vaccines administered.
**Transient; during a flu-like illness.

Supplementary Table S2. Increases in T-cell responses to CEA and/or MUC-1 postvaccination

Patient No.	CEA	MUC-1
1	positive	not tested
6	neg.	positive
8	positive	positive
9	positive	positive
11	neg.	neg.
13	positive	neg.
14	positive	positive
15	positive	neg.
17	neg.	neg.
19	positive	neg.
20	neg.	neg.
21	neg.	neg.
23	positive	neg.
24	neg.	neg.
25	neg.	neg.

Positive denotes increase in specific T cells postvaccination.
Neg. denotes no increase in specific T cells postvaccination.

Supplementary Table S3. Vaccinia and fowlpox antibodies

Patient No.	IgG titers[1] Vaccinia Pre	Vaccinia Post	Fowlpox Pre	Fowlpox Post	Neutralizing titer fowlpox[2]
2	<50	3750	<50	250	Negative
3	250	>6250	<50	850	Negative
4	750	1000	<50	1250	Negative
6	50	>6250	<50	150	Negative
8	750	>6250	<50	5000	Negative
9	<50	3000	<50	150	Negative
11	1250	>6250	<50	125	Negative
12	250	2250	<50	<50	Negative
13	2500	5750	<50	>6250	Negative
14	4250	4250	<50	<50	Negative
15	170	5000	<50	<50	Negative
16	1250	2500	<50	1250	Negative
17	1250	>6250	<50	1250	Negative
19	750	>6250	<50	100	Negative
20	500	6000	<50	1250	Negative
21	750	5750	<50	<50	Negative
22	<50	650	<50	1250	Negative
23	>6250	>6250	<50	<50	Negative
24	6250	>6250	<50	250	Negative
25	850	5000	<50	100	Negative

[1] IgG specific for vaccinia virus and fowlpox virus was determined from patient sera obtained prior to vaccinia prime vaccination (pre) and after 2 fowlpox boosts (post). IgG titers were determined by ELISA as described previously.

[2] Neutralizing antibody titers were determined for fowlpox by reporter gene inhibition assay. Negative: no neutralization at a serum dilution of 1:50.