

Tumor-DNA Based Vaccines Fail to Induce Autoimmune Disease in Mice

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Abstract: Allogeneic cellular cancer vaccines that express tumor antigens specified by tumor-DNA have been found to be effective in the treatment of mice with intracerebral breast cancer, a metastasis model system. The vaccines were prepared by the transfer of genomic DNA from a spontaneously arising adenocarcinoma of the mammary gland into a mouse fibroblast cell line (LM). The immunity in tumor-bearing mice treated by immunization with the DNA-based vaccines was specific for the type of tumor from which the DNA was obtained. It was driven mainly by CD8+ T-cells. Here, we present data indicating that animals receiving the therapeutic vaccines failed to exhibit signs of autoimmunity, as indicated by an examination of various H/E stained organs and tissues including brain for infiltrating inflammatory cells and by the absence of serum anti-nuclear antibody (ANA) in the immunized mice. In addition, tumors derived from the vaccine itself failed to develop in immune-competent tumor-free mice injected with the non-irradiated allogeneic vaccines alone.

Key Words: Immunotherapy, metastasis, breast cancer, autoimmunity.

INTRODUCTION

The underlying rationale for cancer immunotherapy is the capacity of immune cells to recognize cells that express "non-self" cancer associated proteins. Cancer cells that express mutant or dysregulated tumor-associated antigens (TAAs) that are presented to T cells can be targeted by the immune system. Antigenic determinants associated with cancer cells are ideal targets for anti-cancer therapy [1]. Introducing DNA from tumor tissue into antigen presenting cells (APCs) can elicit tumor specific immune responses directed toward an array of undefined tumor-specific antigens (TSAs) that characterize the neoplastic cells.

In this study, the vaccine was prepared by transfer of tumor DNA, which includes multiple genes specifying various TSAs, into LM cells, an immortalized mouse fibroblast cell line. The genes specifying TSAs along with genes specifying normal cellular constituents are expressed by the transfected cells. Vaccines prepared by transfer of genomic DNAs from various cancer cells including spontaneously arising breast carcinoma (SB5b) [2], squamous cell carcinoma to head and neck (SCCHN: KLN 205 and SCCVII cells), have been introduced into fibroblasts. In each instance, immunization of mice with the DNA-based vaccines resulted in tumor-specific immune responses [3-5].

Successful immuno-therapies for intracerebrally metastasizing breast cancer have previously been reported with interleukin-2-secreting fibroblasts [6], various interleukin (IL) secreting syngeneic/allogeneic fibroblasts transfected with DNA from breast cancer cells [7-9], and syngeneic/allogeneic fibroblasts transfected with cDNA from spontaneously arising breast cancer cells [10]. The efficacy was compared in combined immunotherapy with paclitaxel [11]. Enriched cDNA of SB5b cells also was introduced for the intracerebrally metastasized breast cancer vaccine for increased efficacy [10].

T-cell mediated anti tumor immunity appears to be specific and mainly driven by CD8 T-cells. Regulatory T-cells are down-regulated by DNA-based vaccines as indicated by the reduction in the relative numbers of cells that express CD4/CD25, CD62L and Fox P3 [10, 12] in the regressing tumors.

We tested the tumorigenicity of non-irradiated vaccines in immune-competent mice and immune-deficient nude mice to assess the safety of the vaccines. We also monitored weight changes after vaccination to detect toxic effect of the vaccines. The regular vaccination regimen was performed (3 immunizations weekly) and the organs and tissues were recovered for H/E staining and compared with the unvaccinated naïve control for pathologic observations. We also analyzed the sera of immunized mice to determine if anti-nuclear antibodies (ANAs) were present in mice receiving the vaccine. The results indicated that signs of autoimmunity were not found in histological observations of various H/E stained organs and tissues, and that serum anti-

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nuclear antibody above background was undetectable in the immunized mice.

MATERIALS AND METHODS

Experimental Animals, Tumor Cell Lines and Monoclonal Antibodies (mAbs)

Pathogen-free C3H/He, DBA/2J and Nude mice (Nu/J Foxn1^{nu}) mice between 10 to 12 weeks old were from the Jackson Laboratory (Bar Harbor, ME). They were maintained according to NIH Guidelines for the Care and Use of Laboratory Animals.

SB5b cells were a cell line derived from an adenocarcinoma of the mammary gland that arose spontaneously in a C3H/He mouse in our animal colony. SCCVII cells were from squamous carcinoma cell line of C3H/HeJ mouse origin and obtained from the American Type Culture Collection (ATCC). LM fibroblasts, of C3H/He mouse origin, were obtained also from ATCC. Each of the cell types was maintained at 37° in a humidified 7% CO₂/air atmosphere in DMEM (Gibco BRL, Grand Island, NY) supplemented with 10% heat inactivated fetal bovine serum (FBS) (Gibco BRL) (mouse growth medium). ANA test kit (ANA ELISA Kit, Cat. No. 5200) was purchased from Alpha Diagnostic Int. (San Antonio, TX).

Modification of LM Fibroblasts to Secrete IL-2

The fibroblasts were modified to secrete IL-2 before transfection (LM-IL-2 cells) as a means of augmenting their non-specific immunogenic properties, as described previously [13].

Modification of the cytokine-secreting fibroblasts to express H-2K^b-class I-determinants, allogeneic in C3H/He mice. Allogeneic class I-determinants are strong immune adjuvants [14, 15]. To stimulate up take of the vaccine by dendritic cells of the tumor-bearing host, and to ensure rejection, the fibroblasts (H-2^k) were modified to express H-2K^b-determinants, allogeneic in C3H/He mice (LM-IL-2K^b cells), as described previously [16, 2].

Preparation of the DNA-based Cellular Vaccines

LM-IL-2K^b cells were transfected with a genomic DNA with SB5b cells or SCCVII cells, using Lipofectamine 2000 (Invitrogen, Calsbad, CA) to aid DNA uptake. In brief, 2 x 10⁶ LM-IL-2K^b cells were added to four 100mm plates in minimal growth medium (MGM) without antibiotics. Afterward, 30µg of genomic DNA from either of the cell types were mixed with 3 µg pcDNA6/ V5-HisA, a plasmid specifying a gene conferring resistance to blasticidin, in 2ml Opti medium and was mixed with 100 µl Lipofectamine 2000 (Invitrogen, Calsbad, CA), followed by incubation for 20' at RT. 1 ml of the 4ml transfection complex (pcDNA6/V5-HisA/Grb10 DNA and Lipofectamine 2000) was added to each of the four plates and incubated overnight in at 37° in a 7% CO₂/air incubator. The number of plates was expanded to sixteen. The transduced cells were selected in growth medium containing 5 µg /ml blasticidin. The blasticidin-resistant cells were allowed to proliferate for 7 additional days and pooled and maintained as cell lines for use in the experiments (LM-IL-2K^b/SB5b or LM-IL-2K^b /SCCVII respectively). For use as a control, the same procedure was

followed except that the fibroblasts were transfected with pcDNA/V5-HisA alone (LM-IL-2K^b cells). One half of the cell suspension was maintained frozen/viable; the remaining portion was maintained at 37° in a 7% CO₂/air incubator in selection medium. For use as a control, the same procedure was followed except that the LM-IL-2K^b cells were transduced with the "empty" vector pcDNA6/V5-HisA.

Tumor GROWTH in Immuno-deficient and Immuno-Competent Mice

1x10⁶ LM fibroblasts transfected with purified DNA from a breast adeno carcinoma that arose spontaneously in a C3H/He mouse (LMIL-2K^b/SB5b) were subjected to 5000 cGy and injected sc into NU/J Foxn1^{nu} mice. As a control, the same protocol was followed except that the mice were not irradiated before injection. There were three mice in LMIL-2K^b treated group and six mice in LMIL-2K^b/SB5b treated group.

Immunization for H/E Staining and Weight Loss Determination

Female, 10-12 weeks of age DBA/2J, C3H/HeJ mice and nude mice (Nu/J Foxn1^{nu}), from Jackson Laboratory were injected s.c. with syngeneic/allogeneic fibroblasts, transfected with genomic DNA-fragments. Viable 5x10⁶ LM-IL-2K^b, LM-IL-2K^b/SB5b or LM-IL-2K^b/SCCVII cells were injected s.c. on lower left abdomen weekly for three weeks. One week after the last injection, the mice were sacrificed by CO₂ euthanasia and various organs were taken for H/E staining.

ANA ELISA Test

C3H/HeJ mice (female, 10-12 weeks of age, Jackson Laboratory) were injected s.c. with syngeneic/allogeneic fibroblasts, transfected with genomic DNA-fragments from SB5b cells (a breast cancer cell line of C3H/HeJ mouse origin). Viable 5x10⁶ LM-IL-2K^b, LM-IL-2K^b/SB5b cells were injected s.c. on lower left abdomen weekly for three weeks. The mice were weighed and bled through retro-orbital route at day 21, 28 and 42 of the first injection.

ANA test was performed using ANA ELISA Kit (Cat. No. 5200) from Alpha Diagnostic Int. (San Antonio, TX) according to the manufacturer's instruction.

RESULTS

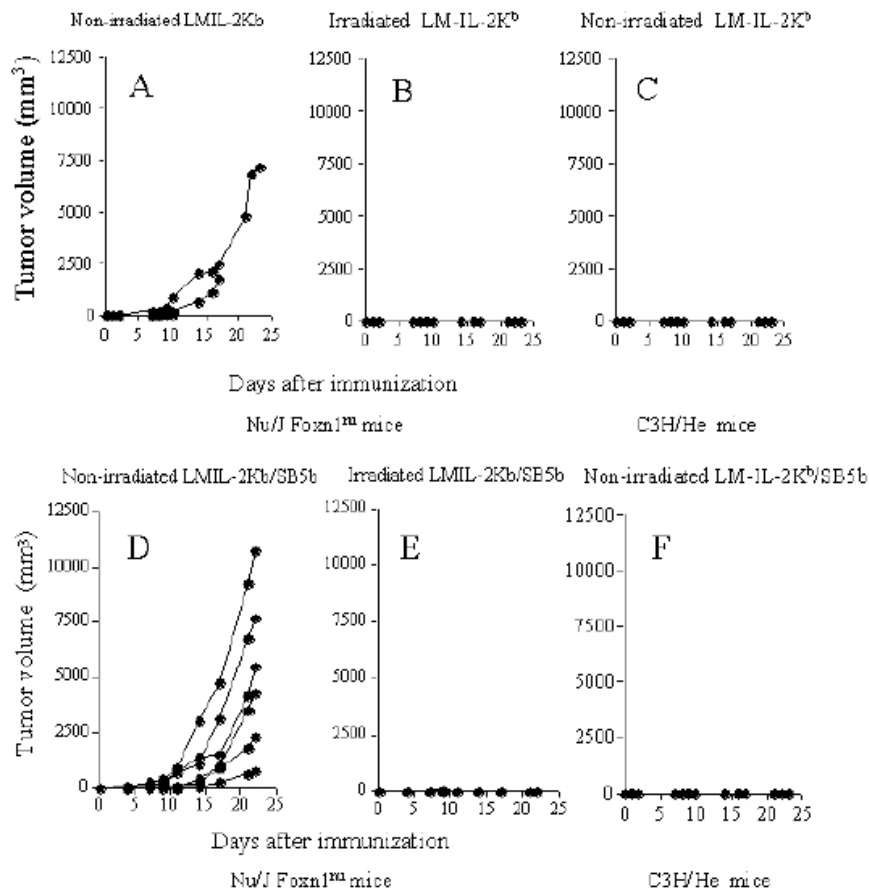
Tumors failed to develop in immune-competent C3H/He mice immunized with viable DNA-transfected cells that express allogeneic MHC-determinants.

The DNA-based vaccine used in this study expressed both H-2^k and H-2^b class I-determinants. C3H/He mice syngeneic with the breast cancer cells express H-2^k but not H-2^b determinants. The vaccine is, therefore, semi allogeneic in C3H/He mice. To determine if tumors derived from the vaccine itself formed in C3H/He mice, tumor-free naïve C3H/He mice were injected s.c. with 1x10⁶ viable vaccine cells. As a control, the same protocol was followed except that the cells were injected into immune-deficient NU/J Foxn1^{nu} mice. The results (Table 1 and Fig. 1) indicated that none of the immune-competent mice developed tumors. Like other allografts, the cells were rejected. In contrast, tumors

Table 1. Tumor Growth in NU/J Foxn1^{nu} Mice Injected with Irradiated IL-2 Secreting Fibroblasts Transfected with DNA from Breast Carcinoma Cells

Vaccines	Tumor growth with Non-irradiated vaccines in Nude mice	Tumor growth with Irradiated vaccines in Nude mice	Tumor growth with Non-irradiated vaccines In C3H/He mice
LMIL-2Kb	3/3 (100%)	0/3 (0%)	0/3 (0%)
LMIL-2Kb/SB5b	6/6 (100%)	0/6 (0%)	0/6 (0%)

1×10^6 LM fibroblasts transfected with purified DNA from a breast adenocarcinoma that arose spontaneously in a C3H/He mouse (LMIL-2Kb/SB5b) were subjected to 5000 cGy and injected sc into NU/J Foxn1^{nu} mice. As a control, the same protocol was followed except that the mice were not irradiated before injection. There were three mice in each group for LMIL-2Kb treated group and six mice in each group for LMIL-2Kb/SB5b treated group.

**Fig. (1). Tumor Growth in NU/J Foxn1^{nu} mice and C3H/He mice injected with irradiated or non-irradiated IL-2 secreting fibroblasts transfected with DNA from breast carcinoma cells.**

Non-irradiated [A] or 5000 cGy irradiated [B] 1×10^6 LM fibroblasts modified to secrete IL-2 and express MHC class I K^b determinants (LMIL-2K^b) were injected sc into NU/J Foxn1^{nu} mice. There were three mice in each group.

1×10^6 LM fibroblasts transfected with purified DNA from a breast adenocarcinoma that arose spontaneously in a C3H/He mouse (LMIL-2K^b/SB5b) were subjected to 5000 cGy and injected sc into NU/J Foxn1^{nu} mice [E]. As a control, the same protocol was followed except that the mice were not irradiated [D] before injection. There were six mice in each group.

5×10^6 LM fibroblasts transfected with purified DNA from a breast adenocarcinoma that arose spontaneously in a C3H/He mouse (LM-IL-2K^b/SB5b) were injected sc into C3H/He mice [F]. As a control, the same protocol was followed except that the mice were not transfected [C]. There were three mice in LM-IL-2K^b/SB5b group.

developed in one hundred percent of the immune-deficient mice injected with an equivalent number of cells.

To determine if X-irradiation of the cells would inhibit tumor formation in immune-deficient mice, the vaccine was subjected to 5000 cGy before it was injected. As a control, the same protocol was followed except that the cells were not irradiated before injection. The results indicated that none of the immune-deficient NU/J Foxn1^{nu} mice injected

with the irradiated vaccines developed tumors. In contrast, all of the immune-deficient mice injected with non irradiated vaccines formed tumors (Table 1 and Fig. 1). There were three mice in LMIL-2K^b treated group and six mice in LMIL-2K^b/SB5b treated group.

Infiltrating inflammatory cells were undetectable in various organs and tissues of mice immunized with the DNA-based vaccines.

Table 2. Infiltrating Inflammatory Cells in Various Organs and Tissues of Mice Receiving DNA-Based Vaccines

Mice: Vaccine	Kidney	Heart	Brain	Liver	Spleen	Duodenum	Thyroid	Lymph Nodes	Leg Muscle
DBA: Naive	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
DBA: LM-IL-2K ^b	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
C3H: Naive	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
C3H:LM-IL-2K ^b /SB5b	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)

Legend: DBA/2J and C3H/HeJ mice (female, 10-12 weeks of age, Jackson Laboratory) were injected i.v. with syngeneic/allogeneic fibroblasts, transfected with genomic DNA-fragments from SB5b cells (a breast cancer cell line of C3H/HeJ mouse origin). After selection, the transfected cells were used to immunize DBA/2J or C3H/He mice as indicated.

Viable 5×10^6 LM-IL-2K^b or LM-IL-2K^b/SB5b cells were injected s.c. on lower left abdomen weekly for three weeks. One week after the last injection, the mice were sacrificed by CO₂ euthanasia and various organs were taken for H/E staining.

Abbreviations: DBA: Naive = non-injected DBA/2J mouse, DBA: LM-IL-2K^b = LM fibroblasts modified to secrete IL-2 and express MHC class I K^b determinants, C3H: Naive = non-injected C3H/HeJ mouse, C3H: LM-IL-2K^b/SB5b = LM fibroblasts modified to secrete IL-2 and express MHC class I K^b determinants transfected with genomic DNA from SB5b cells.

(-) No infiltrating cells, (±) infiltrating cells in < 5% of the organ/tissue, (+) infiltrating cells in 10-20% of the organ/tissue, (++) infiltrating cells in 20-40% of the organ/tissue, (+++) infiltrating cells in 40-80% of the organ/tissue, (++++) infiltrating cells in > 80% of the organ/tissue.

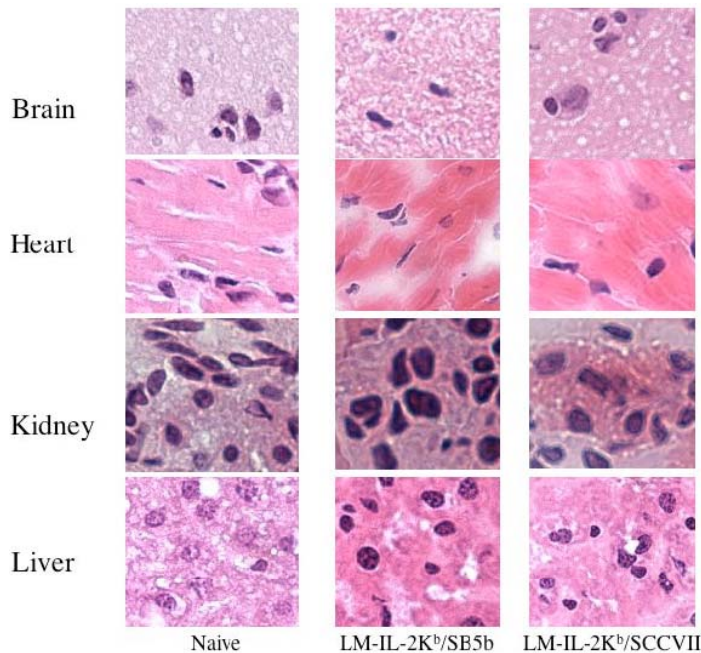


Fig. (2). H/E slides showing no infiltrating inflammatory cells in various organs of mice receiving DNA-based vaccines.

C3H/HeJ mice (female, 10-12 weeks of age, Jackson Laboratory) were injected s.c. with syngeneic/allogeneic fibroblasts, transfected with genomic DNA-fragments from SB5b cells (a breast cancer cell line of C3H/HeJ mouse origin) or SCCVII cells (Squamous carcinoma cell line of C3H/HeJ mouse origin). After selection, the transfected cells were used to immunize C3H/He mice.

Viable 5×10^6 LM-IL-2K^b/SB5b or LM-IL-2 K^b/SCCVII cells were injected s.c. on lower left abdomen weekly for three weeks. One week after the last injection, the mice were sacrificed by CO₂ euthanasia and various organs were taken for H/E staining.

Brain, Heart, Kidney and Liver organs were selected to show H/E staining.

Naive designates organs from non-injected C3H/HeJ mouse.

In addition to TSA, the vast majority of determinants expressed by malignant cells are normal cellular constituents, homologous to the analogous determinants expressed by non malignant cells. Thus, autoimmunity directed toward the constituents of normal cells is a concern. To investigate this question, 5×10^6 viable LM-IL-2K^b, LM-IL-2K^b/SB5b or LM-IL-2K^b/SCCVII cells were injected s.c. on lower left abdomen of DBA/2J and C3H/HeJ mice (female, 10-12

weeks of age, Jackson Laboratory) weekly for three weeks. One week after the last injection, the mice were sacrificed by CO₂ euthanasia. Portions of the brain as well as kidney, heart, liver, spleen, duodenum, thyroid, lymph nodes and muscle were taken for histologic study. The results indicated that the presence of inflammatory cells was no greater in tissues from the immunized mice than those from non immunized naive mice (Table 2 and Fig. 2).

Table 3. Anti-Nuclear Antibodies (ANA) in Modified Fibroblast Vaccine Immunized Mice

	Day 21	Day 28	Day 42
C3H: Naïve 1	(-)	(-)	(-)
C3H: Naïve 2	(-)	(-)	(-)
C3H: LM-IL-2K ^b 1	(-)	(-)	(-)
C3H: LM-IL-2K ^b 2	(+)	(-)	(-)
C3H:LM-IL-2K ^b /SB5b 1	(-)	(-)	(-)
C3H:LM-IL-2K ^b /SB5b 2	(-)	(-)	(-)
C3H:LM-IL-2K ^b /SB5b 3	(-)	(-)	(-)
Positive control from Alpha Diagnostic Int.	(+++)		

Legend : C3H/HeJ mice (female, 10-12 weeks of age, Jackson Laboratory) were injected s.c. with syngeneic/allogeneic fibroblasts, transfected with genomic DNA-fragments from SB5b cells (a breast cancer cell line of C3H/HeJ mouse origin). 5x10⁶ LM-IL-2K^b, LM-IL-2K^b/SB5b cells were injected s.c. on lower left abdomen weekly for three weeks. The mice were weighed and bled through retro-orbital route at day 21, 28 and 42 of the first injection.

ANA test was performed using ANA ELISA Kit (Cat. No. 5200) from Alpha Diagnostic Int. (San Antonio, TX) according to the manufacturer’s instruction.

Abbreviations: C3H: Naïve 1= non-injected C3H/HeJ mouse 1, C3H: Naïve 2 = non-injected C3H/HeJ mouse 2, C3H:LM-IL-2K^b = LM fibroblasts modified to secrete IL-2 and express MHC class I K^b determinants, C3H:LM-IL-2K^b/SB5b = LM fibroblasts modified to secrete IL-2 and express MHC class I K^b determinants transfected with genomic DNA from SB5b cells.

- (-) Negative on anti-nuclear antibody (ANA): specific absorbance at 450nm A < 0.28,
- (+) Positive on anti-nuclear antibody (ANA): specific absorbance at 450nm 1>A_≥0.28
- (++)Positive on anti-nuclear antibody (ANA): specific absorbance at 450nm 1.5>A_≥1
- (+++)+Positive on anti-nuclear antibody (ANA): specific absorbance at 450nm A_≥1.5

Anti-Nuclear Antibody (Positive > 0.28)

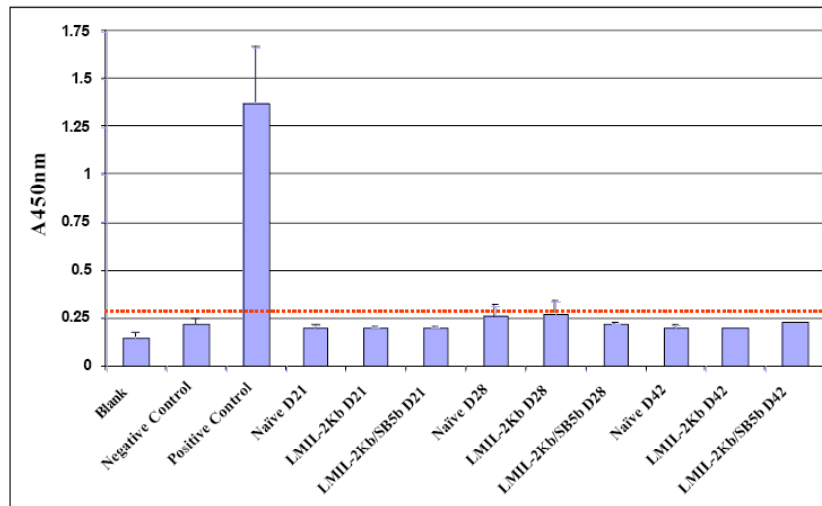


Fig. (3). Anti-Nuclear Antibodies (ANA) in modified fibroblast vaccine immunized mice.

C3H/HeJ mice (female, 10-12 weeks of age, Jackson Laboratory) were injected s.c. with syngeneic/allogeneic fibroblasts, transfected with genomic DNA-fragments from SB5b cells (a breast cancer cell line of C3H/HeJ mouse origin).

Viable 5x10⁶ LM-IL-2K^b, LM-IL-2K^b/SB5b cells were injected s.c. on lower left abdomen weekly for three weeks. The vaccinated mice along with naïve control mice were bled through retro-orbital route at day 21, 28 and 42 of the first injection.

ANA test was performed using ANA ELISA Kit (Cat. No. 5200) from Alpha Diagnostic Int. (San Antonio, TX) according to the manufacturer’s instruction.

There were two mice each in naïve and LM-IL-2K^b control groups and three mice in LM-IL-2K^b/SB5b group.

Anti-nuclear Antibody was Undetectable in the Serum of Vaccinated Mice

To determine if anti nuclear antibody (ANA) formed in mice injected with the vaccines, C3H/HeJ mice (female, 10-12 weeks) were injected s.c. with the syngeneic/allogeneic fibroblasts transfected with genomic DNA-fragments from SB5b cells (a breast cancer cell line of C3H/HeJ mouse ori-

gin) (LM-IL-2K^b/SB5b). 5x10⁶ viable LM-IL-2K^b/SB5b cells or, as a control, non transfected cells (LM-IL-2K^b) were injected s.c. on the lower left abdomen weekly for three weeks. The mice were weighed and bled through retro-orbital route at day 21, 28 and 42 of the first injection. An ELISA test was performed to detect ANA. The results (Table 3 and Fig. 3) failed to reveal the presence of the antibodies above background.

Weight Change After Immunization

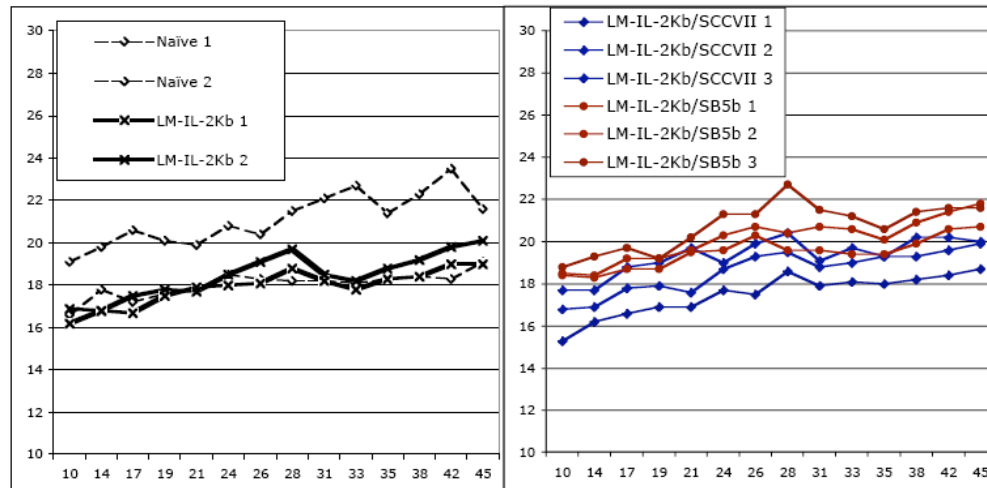


Fig. (4). Weight changes in C3H/He mice injected with IL-2 secreting fibroblasts transfected with DNA from breast carcinoma cells and from squamous carcinoma cells.

Viable 5×10^6 LM fibroblasts transfected with purified DNA from a breast adenocarcinoma that arose spontaneously in a C3H/He mouse (LM-IL-2K^b/SB5b) or from a squamous carcinoma of the head and neck in C3H/He mouse (LM-IL-2K^b/SCCVII) were injected sc into C3H/He mice. As a control, the same protocol was followed except that the mice were not transfected. The mice were weighed every 3-4 days until 45 days. There were two mice in each naïve and non-transfected control groups (Left panel) and three mice in LM-IL-2K^b/SB5b and LM-IL-2K^b/SCCVII experimental groups (Right panel).

Weight Changes in C3H/He Mice Injected with IL-2 Secreting Fibroblasts Transfected with DNA from Breast Carcinoma Cells and from Squamous Carcinoma Cells

To determine if immunization with the vaccines affected the weight of the animals, C3H/HeJ mice (female, 10-12 weeks of age, Jackson Laboratory) were injected s.c. with viable, non irradiated LM-IL-2K^b/SB5b cells. As a control, the same protocol was followed except that the mice were injected with non transfected LM-IL-2Kb cells. As an additional control, a vaccine prepared by transfer of DNA fragments from a squamous carcinoma of the head and neck (LM-IL-2K^b/SCCVII) were substituted for LM-IL-2K^b/SB5b cells. The mice were weighed every 3-4 days until 45 days. The results (Fig. 4) indicated that the weight of the immunized and non immunized mice were not significantly different from each other.

DISCUSSION

The obstacles in immunotherapy of malignant brain tumors by suppression of cell-mediated immunity to disrupt the immune responses were reviewed extensively [17]. Malignant cells, such as breast cancer cells, can also be highly immunosuppressive.

However, many new TAAs have driven novel vaccine and antibody-targeted responses for cancer immunotherapy. Passive immunotherapy, which involves antibodies or toxins without specifically inducing a host antitumor response, active immunotherapy with the tumor-bearing host to elicit an antitumor immune response *in vivo*, or adoptive immunotherapy, which the *ex vivo* expansion of effector cells and return of these effectors to the tumor bearing hosts are three major established routes for immunotherapy. Active immunization is favored for TAA where tolerance is incomplete and

passive immunization can help when the immune repertoire is affected by tolerance or immune suppression [18].

To develop vaccines for active immunotherapy of glioblastoma, a vaccinia virus recombinant expressing inactive form of p53 was prepared. The vaccine induced CD4+ and CD8+ T cells-mediated immune response that protect against subcutaneous challenge with a syngeneic glioblastoma cell line [19]. Protection was also achieved upon vaccination with *in vitro* matured dendritic cells pulsed with the RNA of the GL261 glioma cell line indicating that vaccination can protect against the intracerebral progression of a glioblastoma [20]. To find a stronger and broader immune response than the one that suffices to eradicate peripheral tumors, the RNA of the glioma cell line was screened by RT-PCR and cDNA micro-arrays for differential gene expression in comparison to untransformed brain cells. Tyrosinase related protein 2 (TRP-2) and murine homologue of human melanoma antigen encoding gene element 1 (SMAGE-2) were highly expressed in the glioblastoma cell line. Plasmid DNA vaccines expressing TRP-2 were generated and shown to induce CD8+ T cell responses and protection against intracerebral challenge with the GL261 [21].

Immunotherapies to compensate primary forms of treatment of metastatic breast cancer are well established [22-24]. Nevertheless, not all vaccines are successful, sometimes, tumor progression continues after the beginning of immunotherapy. Immune tolerance to the TAA or the hindrance by the regulatory T cells, can weaken the tumor immunity.

Cutaneous anergy, lymphopenia, impaired antibody production, reduced lymphocyte protein synthesis, and diminished lymphocyte responsiveness, defects in the T-cell compartment are a few example of reported obstacles to be overcome [18].

Attempts to overcome immunity against tumor antigens and efforts to immunize actively against the same antigens can also cause inducing an autoimmune response against the normal central nervous system (CNS). The use of Tregs in preventing autoimmunity and associating immunotherapy may be compounded by employing Treg depletion as an immunotherapeutic adjuvant. However, the adoptive transfer of CNS antigen-specific Tregs into EAE-susceptible mice proved capable of preventing or alleviating the autoimmunity [25]. Recently, subset of histone deacetylase (HDAC) inhibitors have been reported to be beneficial for immunotherapy by targeting FOXP3(+) regulatory T cells [26], and epigenetic modification may improve the future outcome of immunotherapy of cancer.

Our experiment assessing tumorigenicity of non-irradiated vaccines in immuno-competent mice and immune-deficient nude mice along with irradiated control to review the safety of the vaccines are crucial steps for immunotherapy. Evaluating weight changes after vaccination and H/E staining of the organs and tissues, especially brain for observations in infiltrating inflammatory cells can detect adverse effect of the vaccines. We also analyzed immunized mice serum to detect anti-nuclear antibodies (ANAs), which are antibodies against the cell nucleus, which are raised in autoimmune diseases. All negative signs of autoimmunity and toxicity were found in various H/E stained organs and tissues and serum.

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