8.6- TF-Ag, or combinations thereof.

Abstract: Provided are compositions and methods for prophylaxis and/or therapy of cancer. The method involves administering to an individual in need of prophylaxis and/or therapy of cancer a composition which contains tanned mammalian red blood cells complexed with one or more agent(s) that can be a peptide having any of SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4, or asialoglycophorin, or Thomsen-Friedenreich antigen (TF-Ag). Also provided are compositions which contain tanned red blood cells complexed with the peptides, asialoglycophorin, TF-Ag, or combinations thereof.
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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COMPOUNDS AND METHODS OF IMMUNIZATION WITH TUMOR ANTIGENS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. provisional application no. 61/535,185, filed September 15, 2011, the disclosure of which is incorporated herein by reference.

FIELD OF THE INVENTION

[0002] This invention relates generally to the field of compounds and methods for immunization against cancer, and more particularly to the use of use of tumor antigens like TF-antigen to block tumor growth and metastasis.

BACKGROUND OF THE INVENTION

[0003] Tumor-associated carbohydrate antigens (TACA) result from the aberrant glycosylation that is seen with transformation to a tumor cell. The selection of a vaccine target antigen is based not only on the presence of the antigen in a variety of tumor tissues but also on the role this antigen plays in tumor growth and metastasis.

[0004] The tumor-associated carbohydrate Thomsen-Friedenreich antigen (TF-Ag; Galβ1-3GalNAc0C-O-Ser/Thr; the core 1 disaccharide) remains cryptic on normal cell membranes, covered by extended carbohydrate residues preventing exposure of the epitope to the immune system, but is overly expressed on the cell surface of several types of tumor cells, including those of breast, lung, colon, bladder, and prostate, and contributes to cancer cell adhesion and metastasis to sites containing TF-Ag-binding lectins.

[0005] A highly specific IgG₃ monoclonal antibody developed to TF-Ag (JAA-F11) impedes TF-Ag binding to vascular endothelium, blocking a primary metastatic step and providing a survival advantage. In addition, in patients, even low levels of naturally occurring antibodies to TF-Ag appear to improve prognosis, thus it is expected that vaccines generating antibodies towards TF-Ag would be clinically valuable and safe. Unfortunately, vaccinations with protein conjugates of carbohydrate tumor-associated antigens have induced clinically inadequate immune responses, as they are weak immunogens. There is accordingly an ongoing and unmet need for compositions and methods for use in developing an immune response against tumor cells that express TF-Ag. The present invention meets this need.
BRIEF SUMMARY OF THE INVENTION

[0006] The present invention provides compositions and methods for prophylaxis and/or therapy of cancer. The method comprises administering to an individual in need of prophylaxis and/or therapy of cancer a composition comprising tanned mammalian red blood cells complexed with one or more agent(s) selected from: i) a peptide selected from the group consisting of peptide B1 (HHSHKTNLATTGGG; SEQ ID NO:1), CI (YPSLPVVHSRLSGGG; SEQ ID NO:2), D1 (MHPWSGMQVPGGG; SEQ ID NO:3), D2 (HIHGWKSPLSSLGGG; SEQ ID NO:4), and combinations thereof; ii) asialoglycophorin; iii) Thomsen-Friedenreich antigen (TF-Ag); and combinations thereof. The peptides can be provided as a complex with of the peptide and bovine serum albumin (BSA), and may be chemically conjugated to BSA. TF-Ag can also be provided in a in a complex with BSA. The tanned red blood cells are made using conventional techniques which involve exposing the cells to tannic acid. The red blood cells used in the invention are mammalian blood cells, and may be obtained from human or non-human mammals. Practicing the method of the invention can elicit both humoral and cell-mediated immune responses, which can be effective to inhibit tumor growth, and/or inhibit metastasis, and/or extend the life of the individual to whom the compositions of the invention are administered.

[0007] The invention also provides composition comprising an isolated population of tanned red blood cells, wherein the tanned red blood cells are complexed with one or more of peptide B1, CI, D1, D2, asialoglycophorin; TF-Ag, or combinations thereof. Multivalent compositions are also provided. The compositions can comprise a pharmaceutically acceptable carrier and are accordingly considered to be pharmaceutical preparations.

BRIEF DESCRIPTION OF THE FIGURES

[0008] Figure 1 shows the average IL-2 producing cells stimulated with TF-Ag-BSA, divided in bars based on the mouse tested. All values are significantly higher than the control. The results indicate a T cell dependent immune response has been elicited.

[0009] Figure 2 This figure shows the average IL-2 producing cells stimulated with CI-BSA, divided in bars based on the mouse tested. All values are significantly higher than the control.

[0010] Figure 3 This figure shows the average IL-2 producing cells stimulated with asialoglycophorin, divided in bars based on the mouse tested. All values are significantly higher than the control.
Figure 4 shows the average IL-2 producing cells from the non-stimulated mice, divided in bars based on the mouse tested. The first two bars (starting at the left-hand side, Asialoglycophorin, Cl-BSA) have significantly higher IL-2 producing cell numbers as compared to the last bar (PBS control).

Figure 5 shows the average IL-10 producing cells from the mice spleen cells stimulated with TF-Ag-BSA, divided in bars based on the mouse tested. The first two bars (starting at the left-hand side, D2-BSA and Asialoglycophorin) have significantly lower IL-10 producing cell numbers as compared to the third bar (PBS control). IL-10 production is related to specific inhibition of response to the molecule and it is believed that specific inhibition of responses to tumor associated antigens play a role in cancer. These results indicate that the present invention reduces the inhibition response.

Figure 6 shows the average IL-10 producing cells from the mice spleen cells stimulated with B1-BSA, divided in bars based on the mouse tested. The first bar corresponding to the B1-BSA mouse has a significantly higher IL-10 producing cell numbers as compared to the PBS control.

Figure 7 shows the average IL-10 producing cells from the mice spleen cells stimulated with asialoglycophorin, divided in bars based on the mouse tested. The first bar corresponding to the asialoglycophorin mouse had a significantly lower IL-10 producing cell numbers as compared to the PBS control.

Figure 8 shows the average IL-10 producing cells from the mice spleen cells with no stimulation, divided in bars based on the mouse tested. The four bars (D2-BSA, Cl-BSA, TF-Ag-BSA, Asialoglycophorin) correspond to the four mice that have significantly lower number of IL-10 producing mice as compared to the PBS mouse control (last bar).

Figure 9 shows results from vaccination of mice with peptide mimics of TF-Ag, synthetic TF-Ag-BSA, or asialoglycophorin coated on sheep red blood cells. Mice were vaccinated 3 times bi-weekly. Sera were obtained preimmune and post vaccination and analyzed for IgM antibody to TF-Antigen by ELISA. Peptide mimic Cl I had significant increase (means; p<0.05 by Student's t test). The control BSA also had significant increase in IgM.

Figure 10 shows results obtained from mice vaccinated with peptide mimics of TF-Ag, synthetic TF-Ag-BSA, or asialoglycophorin coated on sheep red blood cells. Mice were vaccinated 3 times bi-weekly. Sera were obtained preimmune and post vaccination and analyzed
for IgG antibody to TF-antigen by ELISA. Comparing Pre to Post, peptide mimic Bl, CI, Dl, and D2 as well as TF-Ag groups had significant increase (means; p<0.05 by Student's t test).

[0018] Figure 11 shows results obtained from mice vaccinated with peptide mimics of TF-Ag, synthetic TF-Ag-BSA, or asialoglycophorin coated on sheep red blood cells. Mice were vaccinated 3 times bi-weekly. Week 8: Challenged with 4T1 breast tumor cells. Week 10: Tumors removed. Primary tumor size was significantly smaller in groups immunized with D2 peptide-BSA-tanned red cells, TF-Ag-BSA-tanned cells, asialoglycophorin-tanned cells only.

[0019] Figure 12 provides a graphical depiction of data showing the relationship of IgM optical density in EIA with tumor weight. Briefly, there is (A) no relationship is seen in the control group; a direct relationship is seen in (B) as tumor weight increases with increase in antibody concentration in the carbohydrate group and an indirect relationship as (C) tumor weight decreases with increase in antibody concentration in the peptide group.

[0020] Figure 13 provides a Kaplan Meier analysis showing that the carbohydrate group has a significant survival advantage compared to the control group (p=0.031) while survival for the peptide group was approaching significance (p=0.058).

[0021] Figure 14 shows the percentage of different numbers of lung metastasis for controls (PBS, OVA, BSA), peptides (Bl, C1 and D2) and carbohydrates (TF-Ag and asialoglycophorin).

[0022] Figure 15 provides a graphical representation of data obtained from analysis of mice vaccinated with peptide mimics of TF-Ag, and BSA coated on sheep red blood cells. Mice were vaccinated 3 times bi-weekly. Week 8: Challenged with 4T1 breast tumor cells. Week 10: Tumors removed. Primary tumor size was significantly smaller in the group immunized with D2 peptide-BSA-tanned red cells only (Student's t-test p<0.05).

[0023] Figure 16 summarizes a Kaplan Meier Analysis which shows that the peptide group has a significant survival advantage compared to the control BSA group, p=0.015.

[0024] Figure 17 provides a graphical depiction of data showing the percentage of different numbers of lung metastasis after vaccination with the compositions of the invention.

**DETAILED DESCRIPTION OF THE INVENTION**

[0025] The present invention provides compositions and methods for prophylaxis and/or therapy of cancer. The invention facilitates production of humoral and cell-mediated immune responses against cancer cells that display TF-Ag. We demonstrate using clinically relevant
animal models that performing the method of the invention decreases primary tumor size, inhibits metastasis and increases life span relative to controls.

[0026] The method of the invention comprises administering to an individual in need thereof a composition comprising tanned red blood cells complexed with one or more TF-Ag agents. The TF-Ag agents are selected from: i) a TF-Ag peptide mimic; ii) asialoglycophorin; iii) TF-Ag (each of which can be referred to herein as a "TF-Ag agent"). The peptide TF-Ag mimics are selected from the peptides referred to herein as B1, CI, D1 and D2. The TF-Ag mimicking sequences thereof consist of the following sequences: peptide B1 (HHSHKTLATTPGGG; SEQ ID NO:1), CI (YPSLPVYHSLRSGGG; SEQ ID NO:2), D1 (MHKPWSGHMQVPGGG; SEQ ID NO:3) and D2 (HIHGWSPLSSLGGG; SEQ ID NO:4). In preferred embodiments, the peptides used with the invention are D2, CI, or a combination thereof. In certain embodiments, only one, only two, only three, or all four of the peptides can be present in the composition. In certain embodiments, the only TF-Ag agent in the composition can be asialoglycophorin, or TF-Ag. In various embodiments, the invention provides a multivalent vaccine by either providing tanned RBCs that are complexed with more than one antigen, or mixtures of distinct RBC populations that are each complexed with a single, distinct antigen.


[0028] Each of the peptides can be used in the present invention as part of a conjugated protein, wherein the conjugated protein comprises or consists of the sequence of the peptide and a protein, such as Bovine serum albumin (BSA). Thus, the invention provides in certain embodiments a double carrier system whereby the tanned red blood cells act as a carrier, and
BSA or other protein as a carrier of either a peptide mimic or TF-Ag, the latter of which can also be complexed with the protein. Asialoglycophorin is preferably not used in a complex with BSA. The peptides can be conjugated to BSA using any suitable method and reagents, such as by covalent linkages, including but not necessarily limited to covalent bonds between functionalized amino acids, disulfides, and the like. The amino acid sequence of BSA is known in the art. In one embodiment, the BSA is mature BSA which consists of 583 amino acids.

[0029] The compositions of the invention comprise or consist of red blood cells that have been complexed with one or more TF-Ag agents which constitute the TF-Ag peptide mimics; asialoglycophorin; and TF-Ag (each of which can be referred to herein as a "TF-Ag agent"). The TF-Ag agent(s) can be complexed with the red blood cells in a variety of ways, but in general are non-covalently associated with the surface of the red blood cells. The red blood cells can be any mammalian red blood cells. In one embodiment, the red blood cells are human red blood cells. In other embodiments, the red blood cells are non-human mammalian red blood cells, such as red blood cells obtained from sheep. A wide variety of techniques, reagents and devices are available for obtaining red blood cells for use in the compositions and method of the invention.

[0030] To prepare compositions used in the invention, the red blood cells can be modified to become so-called "tanned" red blood cells, which are red blood cells that have been exposed to tannic acid. Suitable protocols for tanning red blood cells are known in the art from, for example, hemagglutination assays. A general protocol for making tanned red blood cells and complexing TF-Ag agents with it is as follows.

[0031] Prepare a tannic acid stock, for example 10mg/ml in water which can be used to make a final working solution (1:5000) of tannic acid in a suitable buffer, such as phosphate buffered saline (PBS) at pH 6.4. Tanning can be performed by washing red blood cells with, for example, saline. After washing, the red blood cells can be re-suspended in a suitable buffer, such as PBS, at pH 6.4 for a 2% cell suspension. Equal volumes of 1:5000 tannic acid and a 2% suspension of red blood cells can be mixed and incubated at, for example, in 37 °C water-bath for 30 minutes. To obtain tanned red blood cells, the suspension that has been exposed to the tannic acid can be centrifuged and preferably washed several times with, for example, saline. The tanned red blood cells can then be re-suspended in suitable volume of a suitable buffer, for example 15mls of PBS at pH 6.4, to obtain a 2% suspension of tanned red blood cells, which can be prepared, for
example, by pelleint cells to 0.2 ml in a suitable container and adding a buffer, such as PBS, to 10 ml, to yield a 2% suspension.

[0032] In general, complexing the TF-Ag agent with tanned red blood cells can be performed using any suitable technique. In one embodiment, equal volumes of 2% tanned cells and the antigen(s) to be coated at, for instance, 10µg/ml in PBS pH 6.4, can be prepared. These can be mixed and incubated for a period of time, at for example at 37°C for 30 minutes. The tanned red blood cells can be centrifuged and washed several times, such as with saline. The cells can be re-suspend in a volume of a sterile buffer to have, for example, a concentration of 2% tanned cells coated with 10µg/ml of antigen.

[0033] Compositions of the invention can comprise or consist of tanned red blood cells complexed with one or more TG-Ag agents of the invention. In various embodiments, the complexed red blood cells comprise from 50% to 100%, inclusive, and including all integers to the first decimal place there between, of the cells in the composition.

[0034] In certain embodiments, the compositions provided by the invention comprise or consist of: tanned red blood cells complexed with peptide B1 (HISHKTNLATTPGGG; SEQ ID NO:1), or peptide C1 (YPSLPVYHSLRSGGG; SEQ ID NO:2), or peptide D1 (MHKPWSGHMQVPGGG; SEQ ID NO:3) or peptide D2 (HIHGWKPSLSLGGG; SEQ ID NO:4), or TF-Ag, or asialoglycophorin, or a combination thereof. The compositions can be prepared such that the only type of cells in the composition are red blood cells, and thus the cellular component of the composition can consist of tanned red blood cells, all or some of which are complexed with one or more TF-Ag agent(s). The composition can include other agents, such as adjuvants or cytokines which are intended to improve the immune response against TF-Ag bearing cells.

[0035] Tanned red blood cells complexed with one or more TF-Ag agents of the invention can be provided as a pharmaceutical preparation. Thus, the tanned red blood cells complexed with one or more of the TG-Ag agents can be mixed with at least one pharmaceutically acceptable carrier, which can be provided in a pharmaceutically acceptable buffer. Some examples of pharmaceutically acceptable carriers can be found in: Remington: The Science and Practice of Pharmacy (2005) 21st Edition, Philadelphia, PA. Lippincott Williams & Wilkins.

[0036] The invention is expected to be suitable for inhibition of growth of all solid tumor of the type called adenocarcinoma, and for inhibition of metastasis from such tumors. In non-
limiting examples, the invention provides for treating an individual who has been diagnosed with, is suspected of having, or is at risk for such a cancer. In particular embodiments, the cancer is any TF Ag+ cancer. In various embodiments, the cancer can include is not limited to, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, head and neck cancer, papillary adenocarcinomas, cystadenocarcinoma, mcervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, and bladder carcinoma.

Various methods known to those skilled in the art may be used to introduce the compositions of the invention to an individual. These methods include but are not limited to intracranial, intrathecal, intradermal, intramuscular, intraperitoneal, intravenous, and subcutaneous routes.

It will be recognized by those of skill in the art that the form and character of the particular dosing regime employed in the method of the invention will be dictated by the route of administration and other well-known variables, such as rate of red blood cell clearance in the individual, the size, age, gender and overall health of the individual, and the stage, type and location of the particular cancer being treated. Based on such criteria, one skilled in the art can determine an amount of any of the compositions described herein that will be effective to inhibit tumor growth and/or metastasis for any particular individual.

In one embodiment, the invention elicits an enhanced T cell response against TF-Ag, important because carbohydrates are normally T-independent antigens. The enhanced T cell response can include but is not necessarily limited to an increase in T cells that exhibit cytotoxic activity against cells that bear TF-Ag, or T cells that exhibit enhanced sustenance and/or antigen-recall responses to the antigen, or an increase of the amount and/or activity of effector T cells or helper T cells that are specific for the antigen, or combinations of the foregoing types of cell mediated immune responses. The T cell response elicited by the method of the invention may be accompanied by beneficial changes in humoral and/or innate immune responses. In one embodiment, an enhanced immune response can be evidenced by an inhibition of the growth of tumor cells that express TF-Ag in the individual, and/or by a prolongation of the survival of the individual, and or by an inhibition of metastasis in the individual.

The method of the invention can be performed in conjunction with conventional anti-cancer therapies. Such therapies can include but are not limited to chemotherapies, surgical
interventions, and radiation therapy. The compositions of the invention could be administered prior to, concurrently, or subsequent to such anti-cancer therapies.

[0041] The invention is illustrated by the following Examples which are intended to demonstrate but not limit the invention.

EXAMPLE 1

[0042] This Example provides a description of enzyme-linked immunosorbent spot (ELISPot) assays performed using tanned red blood cells complexed with TF-Ag agents conjugated to BSA.

[0043] The ELISpot procedure is as follows.

[0044] Six mice were chosen for the ELISpot experiment using samples obtained over a three day period. All of the mice one year, eight months and seven days old when they were sacrificed for the experiment. Six mice that were chosen for the experiment were designated #268 asialoglycophorin on tanned red blood cells, #256 TF-Ag-BSA on tanned red blood cells, #521 Cl-BSA on tanned red blood cells, #577 Bl-BSA on tanned red blood cells, #502 D2-BSA on tanned red cells and #552 PBS. Each of these mice was previously vaccinated with 2ug of antigen LP, (6-13-11, 6-27-11, 7-11-11). Ten days before the ELISpot experiment was performed the mice were vaccinated again to stimulate the spleen cells in vivo.

[0045] On day 1 the ELISpot plates were coated. The IgG ELISpot plate was coated with TF-Ag-BSA (1.25ug/ml), asialoglycophorin (5ug/ml), Cl-BSA (10ug/ml), Bl-BSA (10ug/ml), D2-BSA(10ug/ml), BSA(10ug/ml). Both ELISpot plates and western blot membranes are composed of PVDF. The IL-2 plate was coated as per manufactures instructions with purified anti-mouse IL-2 at 5ug/ml suspended in sterile PBS pH 7.2. The IL-10 plate was coated also following the manufactures instructions with 5ug/ml of purified anti-mouse IL-10 suspended in sterile PBS pH 7.2. IL-2 was chosen as an indicator of a T-dependent response, IL-10 was selected to determine if there was a decrease in the regulatory T cell activity.

[0046] On day 2 the mice spleens were removed, washed and suspended at concentrations of 5x10^6 cells/ml in RPMI 1640 media supplemented with 10% FCS, 1% penicillin/streptomycin, and 1% percent L-glutamine. Two hours prior to the addition of the mice spleen cells, the ELISpot plates were blocked with a 2% fish gelatin blocking solution (fish gelatin in 1x sterile PBS). The 2% fish gelatin blocking solution was equivalent to using a 10% BSA blocking solution. A BSA blocking solution not used because the vaccine antigens were
attached to BSA. For the IgG ELISPOT plate, 5x10^5 of mice spleen cells were added to the wells. The #256 TF-Ag-BSA mouse's spleen cells were added to triplicate wells with TF-Ag-BSA coating, D2-BSA coating, asialoglycophorin coating and BSA coating. The #268 asialoglycophorin mouse's spleen cells were added in triplicate wells with TF-Ag-BSA coating, D2-BSA coating, asialoglycophorin coating, and BSA coating. The #521 Cl-BSA mouse's spleen cells were added in triplicate to wells coated with TF-Ag-BSA, Cl-BSA, asialoglycophorin and BSA. #577 Bl-BSA mouse's spleen cells were added in triplicate to wells coated with TF-Ag-BSA, Bl-BSA, asialoglycophorin and BSA. #502 D2-BSA mouse's spleen cells were added in triplicate to wells coated with TF-Ag-BSA, D2-BSA, asialoglycophorin and BSA. The negative control mouse #552 PBS's spleen cells were added in triplicate to wells with all of the coatings. In two wells for each coating condition only media and reagents were added to assure that the spots created by the mice were true spots. Also in two wells for each coating monoclonal antibody JAA-F1 1 was added at 2.5μg/ml to spleen cells from #552 PBS mouse to act as a positive control.

[0047] For the IL-2 and IL-10 ELISPOT plates 5x10^5 spleen cells were added to each well coated with capture antibody. For the #256 TF-Ag-BSA mouse spleen cells were added to wells and stimulated with lOug/ml of TF-Ag-BSA, D2-BSA or had no stimulant added. Each condition was performed in triplicate wells. The #268 asialoglycophorin mouse's spleen cells were stimulated with lOug/ml of TF-Ag-BSA, asialoglycophorin or no stimulation was added. The #502 D2-BSA mouse's spleen cells were stimulated at lOug/ml with TF-Ag-BSA, D2-BSA or no stimulation was added. The #521 Cl-BSA mouse's spleen cells were stimulated at lOug/ml with TF-Ag-BSA, Cl-BSA or no stimulation was added. The #577 Bl-BSA mouse's spleen cells were stimulated at lOug/ml with TF-Ag-BSA, Bl-BSA and no stimulation was added. The #552 PBS mouse's spleen cells were stimulated with lOug/ml of TF-Ag-BSA, D2-BSA, asialoglycophorin, Cl-BSA, Bl-BSA or no stimulation was added. For both the IL-2 and IL-10 ELISPOT plates the mice spleens were stimulated and plated as mentioned. The positive control for the IL-2 and IL-10 ELISPOT plates that was used was spleen cells from the #552 PBS mouse stimulated with lug/ml of concanavalin a. Three wells per plate contained only media and reagents, to ensure the spots formed were true spots, rather than reagent artifacts.

[0048] After 18 and a half hours of incubation in 37 degrees Celsius five percent CO_2 incubator the ELISPOT plates were removed washed multiple times, secondary antibody
conjugated to biotin was added, then enzyme conjugated to Streptavidin was added followed by substrate. Each spot that developed in the wells corresponded to one cell secreting the particular analyte. The results were as follows.

[0049]  \[ \text{IL}^2 \]

[0050] **Mice stimulated with TF-Ag-BSA**

[0051] ANOVA results indicate that there is a difference in the mean number of IL-2 producing cells stimulated with TF-Ag-BSA demonstrated in the ELISPOT assay according to the form of vaccination given to Balb/c mice \( F(5,12) = 27.728, \ p < 0.0005 \). According to the Tukey HSD multiple comparison procedure with a significance level of 0.05, results indicate that the mean number of IL-2 producing cells from mice stimulated in vitro with TF-Ag-BSA and vaccinated previously with TF-Ag-BSA on tanned red blood cells, Bl-BSA on tanned red blood cells, D2-BSA on tanned red blood cells, Cl-BSA on tanned red blood cells, and asialoglycophorin on tanned red blood cells are significantly higher than IL-2 producing cells from mice vaccinated previously with PBS (negative control) and stimulated with TF-Ag-BSA.

In other words all of the vaccinated groups that were stimulated in vitro with TF-Ag-BSA had a significantly higher number of IL-2 producing cells as compared to the mouse who only received PBS. Stimulation of the mice with TF-Ag-BSA in the ELISPOT simulates the reaction that the mice would have if they encountered tumors bearing TF-Ag in vivo. These results indicate that both the carbohydrate and peptide mimic versions of the TF-Ag vaccine produce T-Cell involvement based on testing of one mouse from each group. To perform ANOVA analysis the IL-2 spot numbers from each of the triplicate wells were used to get an \( n \) of three.

[0052] ANOVA analysis was also performed on the mice's spleen cells stimulated in vitro with Cl-BSA. The ANOVA results indicate that the spleen cells from the mouse previously vaccinated with Cl-BSA have a significantly higher number of IL-2 producing cells as compared to the spleen cells from the mouse previously vaccinated with PBS \( F(1,4) = 13.545, \ p = 0.021 \). The #521 mouse (previously vaccinated with Cl-BSA) appears to have increased IL-2 producing cells which indicates that the peptide mimic vaccine was producing T-cell involvement. This result paired with the data found from the Cl-BSA vaccinated mouse stimulated with TF-Ag-BSA indicate that the Cl-BSA peptide mimic reacts very similarly to TF-Ag-BSA in that they both produce IL-2 production.

[0053] **Mice stimulated with asialoglycophorin**
ANOVA analysis was also performed on the mice's spleen cells stimulated in vitro with asialoglycophorin. The ANOVA results indicate that the spleen cells from the mouse previously vaccinated with asialoglycophorin have a significantly higher number of IL-2 producing cells as compared to the spleen cells from the mouse previously vaccinated with PBS. F(1,4)= 12.701, p=0.024. The multiple comparison procedure could not be performed on this data since only two mice were stimulated with asialoglycophorin in the ELISPOT experiment. This data paired with the data that the #268 asialoglycophorin mouse had a significantly higher IL-2 producing cell population when stimulated with TF-Ag-BSA indicates that a mouse vaccinated with asialoglycophorin on tanned red blood cells will not only produce IL-2 when boosted with the vaccine but will also produce IL-2 when exposed to the antigen found on tumors.

Mice not stimulated

For the mice spleen cells that were not stimulated ANOVA analysis was performed. Our ANOVA results indicate that there is a difference in the mean number of IL-2 producing cells that were not stimulated in the ELISPOT assay according to the form of vaccination given to Balb/c mice F(5,11)=173.5,  p<0.0005. According to the Tukey HSD multiple comparison procedure with a significance level of 0.05, results indicate that the mean number of IL-2 producing cells from mice not stimulated in vitro and vaccinated previously with Cl-BSA on tanned red blood cells, and asialoglycophorin on tanned red blood cells are significantly higher than IL-2 producing cells from mice vaccinated with the other versions of the vaccines and with PBS (negative control) that were not stimulated in vitro.

These results suggest that the #521 Cl-BSA mouse and #268 asialoglycophorin mouse were more stimulated in vivo than any of the other mice. This in vivo stimulation may be reflected in the increased in IL-2 production when stimulated with Cl-BSA and TF-Ag-BSA for the #521-Cl-BSA mouse. This also applies to the #268 asialoglycophorin mouse with TF-Ag-BSA stimulation and asialoglycophorin stimulation. The table below shows the data for the Cl-BSA mouse. For the Cl-BSA mouse not stimulated in vitro the average number of cells producing IL-2 is 35. But for the same mouse stimulated with TF-Ag-BSA there are only 18 cells producing IL-2. This could be because the stimulation itself may have hindered the mice from producing IL-2, or the way the antigens were suspended may have decreased the production of IL-2.
Table 1 shows the average IL-2 producing cells for the #521 mouse, based on stimulation. This table also compares the number of IL-2 producing cells with the control PBS mouse and gives a representation of how many times greater the CI-BSA mouse's number of IL-2 producing cell is greater than the control mouse.

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Stimulation</th>
<th>IL-2 Producing cells</th>
<th>Comparison to Control Mouse IL-2 Producing cell number</th>
<th>Times spot number is greater than control</th>
</tr>
</thead>
<tbody>
<tr>
<td>#521 C1-BSA</td>
<td>TF-Ag-BSA</td>
<td>18</td>
<td>&gt; (3)</td>
<td>+6.00</td>
</tr>
<tr>
<td>#521 C1-BSA</td>
<td>CI-BSA</td>
<td>48</td>
<td>&gt; (9)</td>
<td>+5.33</td>
</tr>
<tr>
<td>#521 C1-BSA</td>
<td>None</td>
<td>35</td>
<td>&gt; (7)</td>
<td>+5.00</td>
</tr>
</tbody>
</table>

For the #268 asialoglycophorin mouse the non-stimulated IL-2 producing cell average is higher than both the TF-Ag-BSA stimulated and asialoglycophorin stimulation, as shown in the table below. This also applies to the CI-BSA mouse data.

Table 2 shows the average number of IL-2 producing spleen cells from #268 asialoglycophorin mouse based on stimulation. It also compares the number of IL-2 producing cells to the control mouse and states how many times higher the number of IL-2 producing spleen cells form #268 mouse is as compared to the PBS mouse.

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Stimulation</th>
<th>Average Number of Spots</th>
<th>Comparison to Control Mouse Spot Number</th>
<th>Times spot number is greater than control</th>
</tr>
</thead>
<tbody>
<tr>
<td>#268 Asialo</td>
<td>TF-Ag-BSA</td>
<td>72</td>
<td>&gt; (3)</td>
<td>+24</td>
</tr>
<tr>
<td>#268 Asialo</td>
<td>Asialoglycophorin</td>
<td>38</td>
<td>&gt; (12)</td>
<td>+3.17</td>
</tr>
<tr>
<td>#268 Asialo</td>
<td>None</td>
<td>86</td>
<td>&gt; (7)</td>
<td>+12.29</td>
</tr>
</tbody>
</table>

IL-10

Mice stimulated with TF-Ag-BSA

ANOVA results indicate that there is a difference in the mean number of IL-10 producing cells stimulated with TF-Ag-BSA demonstrated in the ELISpot assay according to the form of vaccination given to Balb/c mice F(3,8)=23.029 p<0.0005. According to the Tukey HSD multiple comparison procedure with a significance level of 0.05, results indicate that the mean number of IL-10 producing cells from mice vaccinated with D2-BSA on tanned red blood
cells, and asialoglycophorin on tanned red blood cells are significantly different than IL-10 producing cells from mice vaccinated previously with PBS (negative control) and stimulated with TF-Ag-BSA. The mouse vaccinated with D2-BSA had a significantly lower number of IL-10 producing cells as compared to the spleen cells from the mouse vaccinated with PBS.

Whereas the mouse vaccinated with asialoglycophorin has a significantly lower IL-10 spot number as compared to the mouse vaccinated with PBS. The results for the asialoglycophorin mouse correlate with the results from the asialoglycophorin mouse stimulated with TF-Ag-BSA and measured for IL-2 production. This is consistent with the observation that, compared to the controls the IL-2 production was significantly higher and the IL-10 production was significantly lower because IL-10 has an inhibitory effect. The results for the D2-BSA mouse follow the same trend as the asialoglycophorin mouse's results because there is a significantly higher number of IL-2 producing cells and a significantly lower number of IL-10 producing cells as compared to the control. To perform ANOVA analysis the IL-2 spot numbers from each of the triplicate wells were used to get an n of three.

Figure 5. This figure shows the average IL-10 producing cells from the mice spleen cells stimulated with TF-Ag-BSA, divided in bars based on the mouse tested. The first two bars (starting at the left-hand side, D2-BSA and Asialoglycophorin) have significantly lower IL-10 producing cell numbers as compared to the third bar (PBS control).

ANOVA analysis showed no significant difference in IL-10 producing cell number based on method of previous vaccination when spleen cells were stimulated in vitro with Cl-BSA.

ANOVA analysis was also performed on the mice's spleen cells stimulated in vitro with Bl-BSA. The ANOVA results indicate that the spleen cells from the mouse previously vaccinated with Bl-BSA have a significantly higher number of IL-10 producing cells as compared to the spleen cells from the mouse previously vaccinated with PBS F(1,3)= 16.459, p=0.027.

ANOVA analysis was also performed on the mice's spleen cells stimulated in vitro with asialoglycophorin. The ANOVA results indicate that the spleen cells from the mouse
previously vaccinated with asialoglycophorin have a significantly higher number of IL-10 producing cells as compared to the spleen cells from the mouse previously vaccinated with PBS F(1,4)= 127.268, p>0.0005. The multiple comparison procedure could not be performed on this data since only two mice were stimulated with asialoglycophorin in the ELISPOT experiment.

This result was unexpected because there was a significant increase in IL-2 production for the asialoglycophorin mouse stimulated with asialoglycophorin, but we predicted a lower number of IL-10 producing cells, since IL-10 is an immune inhibitory cytokine.

Mice not stimulated

For the mice spleen cells that were not stimulated and ANOVA analysis was performed the ANOVA results indicate that there is a difference in the mean number of IL-10 producing cells that were not stimulated in the ELISPOT assay according to the form of vaccination given to Balb/c mice F(1,3)=16.459, p<0.027. According to the Tukey HSD multiple comparison procedure with a significance level of 0.05, results indicate that the mean number of IL-10 producing cells from mice not stimulated in vitro and vaccinated previously with Cl-BSA on tanned red blood cells, and asialoglycophorin on tanned red blood cells, D2-BSA on tanned red blood cells, TF-Ag-BSA on tanned red blood cells, Bl-BSA on tanned red blood cells (all other groups) are significantly lower than IL-10 producing cells from mice vaccinated with PBS (negative control) that were not stimulated in vitro. Stated differently, the #552 PBS mouse was producing more IL-10 as compared to all of the other mice from the different vaccine groups.

IgG

To obtain the average IgG spot number for the mice the spot numbers were corrected for response to BSA content. This was done by taking the average number of spots from the mouse spleen cells added to wells coated with TF-Ag-BSA, D2-BSA, Bl-BSA or Cl-BSA and subtracting it from their corresponding spot number BSA. This eliminated extraneous spots that were believed to be the result of mice spleen cells producing antibody to the BSA portion of the antigen. We then compared the number of IgG producing cells per each well coating to the control PBS mouse on the corresponding well coating.

Table 3 shows the number of IgG producing cells for the #521 Cl-BSA mouse (the number of IgG producing cells as compared with three plate coatings). There is an increase as compared to the control mouse for only the cells that produced IgG that reacts with the Cl-BSA
coating. This suggests that the Cl-BSA mouse produces an IgG response when stimulated with the same antigen that it was vaccinated.

Table 3

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Plate Coating</th>
<th>Average Number IgG producing cells (BSA Corrected)</th>
<th>Comparison to Control Mouse IgG producing cell Number</th>
<th>Times number of IgG producing cells is greater than control</th>
</tr>
</thead>
<tbody>
<tr>
<td>#521 C1-BSA</td>
<td>TF-Ag-BSA</td>
<td>86</td>
<td>&lt; (124)</td>
<td>0.69</td>
</tr>
<tr>
<td>#521 C1-BSA</td>
<td>C1-BSA</td>
<td>140</td>
<td>&gt; (114)</td>
<td>+1.23</td>
</tr>
<tr>
<td>#521 C1-BSA</td>
<td>None</td>
<td>21</td>
<td>&lt; (91)</td>
<td>0.23</td>
</tr>
</tbody>
</table>

[0076] For the #577 Bl-BSA mouse the number of cells producing IgG for all of the plate conditions was not higher than the control mouse.

[0077] The #502 D2-BSA mouse's spleen cells when added to wells with D2-BSA and asialoglycophorin coating produced had more IgG producing cells than the control mouse. This indicates that the antibodies made against the asialoglycophorin could simulate an in vivo response against the tumors. Table 4 shows the number of IgG producing cells as compared with the three plate coatings.

Table 4

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Plate Coating</th>
<th>Average Number IgG producing cells(BSA Corrected)</th>
<th>Comparison to Control Mouse IgG producing cell number</th>
<th>Times IgG producing cell number is greater than control</th>
</tr>
</thead>
<tbody>
<tr>
<td>#502 D2-BSA</td>
<td>TF-Ag-BSA</td>
<td>2</td>
<td>&lt; (7)</td>
<td>0.28</td>
</tr>
<tr>
<td>#502 D2-BSA</td>
<td>D2-BSA</td>
<td>17</td>
<td>&gt; (16)</td>
<td>+1.06</td>
</tr>
<tr>
<td>#502 D2-BSA</td>
<td>Asialoglycophorin</td>
<td>4</td>
<td>&gt; (3)</td>
<td>+1.33</td>
</tr>
</tbody>
</table>

[0078] The asialoglycophorin vaccinated mouse #268 mouse produced a higher number of IgG producing cells when plated in each of the well coatings. These results suggest that for this mouse the vaccination and an in vivo tumor may possibly elicit the production of IgG. Table 5 shows the number of IgG producing cells as compared with the three plate coatings.
The mouse vaccinated with the TF-Ag-BSA also produced a higher number of IgG producing cells on all of the well coatings. These results may also suggest for this mouse the vaccination and in vivo tumor may possibly elicit the production of IgG. Table 6 shows the number of IgG producing cells as compared with the three plate coatings.

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Plate Coating</th>
<th>Average Number of Spots (BSA Corrected)</th>
<th>Comparison to Control Mouse Spot Number</th>
<th>Times spot number is greater than control</th>
</tr>
</thead>
<tbody>
<tr>
<td>#268 Asialo</td>
<td>TF-Ag-BSA</td>
<td>17</td>
<td>&gt; (7)</td>
<td>+2.43</td>
</tr>
<tr>
<td>#268 Asialo</td>
<td>D2-BSA</td>
<td>59</td>
<td>&gt; (16)</td>
<td>+3.69</td>
</tr>
<tr>
<td>#268 Asialo</td>
<td>Asialoglycophorin</td>
<td>10</td>
<td>&gt; (3)</td>
<td>+3.33</td>
</tr>
</tbody>
</table>

The experiments described in this Example were designed to determine the effect of immunization with peptide mimics of TF-Ag or other constructs of TF-Ag on survival in 11 month old mice with TF-Ag bearing metastatic tumors and involved immunization of TF-Ag peptide mimics and TF-Ag containing constructs attached to tanned red blood cells to Balb/c female mice. It demonstrates that the invention decreases primary tumor size and inhibits metastasis.
Experimental Design:

**Mice immunization**. See Table 7 for immunization protocol. Briefly, the immunization protocol included one primary vaccination plus 3 boosters (every 2 weeks); 4 blood draws (one pre- and 3 post-vaccination). Four different TF-Ag peptide mimics (B1-, C1-, D1-, D2-) attached to BSA, TF-Ag linked to BSA and asialoglycophorin were coated onto tanned sheep red blood cells. Six groups of female balb/c mice (5 per group; 11 months old) were immunized intraperitoneally 3 times biweekly with these different vaccines. Three groups of control animals were vaccinated with Ova peptide, BSA peptide and PBS coated onto tanned sheep red blood cells. Three additional mice were vaccinated intradermally with asialoglycophorin alone. Pre-immune and post vaccination sera obtained from mice were tested for antibody production by ELISA.

Table 7

<table>
<thead>
<tr>
<th>Vaccine</th>
<th># Mice</th>
<th>Vaccine ng/mouse</th>
<th>Primary Vac. Week 0</th>
<th>Boost 1 0.2 ml L.P.</th>
<th>Boost 2 Week 2</th>
<th>Boost 3 Week 4</th>
<th>Boost 3 Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Ova peptide-BSA-tanned red cells</td>
<td>5</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 PBS-BSA-tanned red cells</td>
<td>5</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 B1 peptide-BSA-tanned red cells</td>
<td>5</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 C1 peptide-BSA-tanned red cells</td>
<td>5</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 D1 peptide-BSA-tanned red cells</td>
<td>5</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 D2 peptide-BSA-tanned red cells</td>
<td>5</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 TF-Ag –BSA-tanned red cells</td>
<td>5</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 Asialoglycophorin – tanned red cells</td>
<td>5</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 PBS</td>
<td>5</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Asialoglycophorin (Intradermal)</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Tumor challenge of mice.** The mice were challenged with \(2.5 \times 10^4\) 4T1 breast tumor cells into the mammary gland. The primary tumors were removed and weighed. Tumor outcome was measured by counting the number of lung metastasis in each group of mice. The survival time was recorded and analyzed by Kaplan-Meier survival analysis.

**Results.** No significant difference between the anti-TF-Ag IgM nor the IgG levels of controls versus the test groups was seen although there was a significant difference between the preimmune and the post-vaccination groups (Figure 9 and Figure 10). Tumor challenge showed a statistically significant difference (p value=0.000484 by ANOVA) in tumor weights in (three) of the test groups in comparison with that of the control animals (Figure 11).
When antibody levels were compared with tumor weight, there was i) no correlation between antibody level with tumor weight in the control groups; ii) a positive correlation in the peptide-BSA-tanned cell groups; and iii) a negative correlation in the carbohydrate-tanned cell groups and intradermal group (Figure 12).

Kaplan Meier survival analysis showed that the carbohydrate groups have a significant survival advantage compared to the control groups (p=0.031) whereas for the peptide groups, the survival advantage was approaching significance (p=0.058) (Figure 13).

Analysis of lung metastases indicates that is a significant difference in the number of lung metastases between the test and control groups (Figure 14).

EXAMPLE 3

This Example is similar to Example 2 but was performed with 6-7 month old mice. It demonstrates that immunization of TF-Ag peptide mimics and TF-Ag containing constructs attached to tanned red blood cells to Balb/c female mice decreases primary tumor size and reduces metastasis.

Experimental Design:

Mice immunization The experiments described in Example 2 were essentially repeated using younger female Balb/c mice (6 - 7 months old). D2-peptide mimic attached to BSA, CI-peptide linked to BSA, and BSA peptide were coated onto tanned sheep red blood cells. Three groups of female balb/c mice (6 for CI, 5 for both D2 and BSA group) were immunized intraperitoneally 3 times biweekly with these different vaccines. Pre-immune and post vaccination sera obtained from mice were tested for antibody production by ELISA.

Tumor challenge of mice The mice were challenged with 2.5 x 10^4 4T1 breast tumor cells into the mammary gland. The primary tumors were removed and weighed. Tumor outcome was measured by counting the number of lung metastasis in each group of mice. The survival time was recorded and analyzed by Kaplan-Meier survival analysis.

Results There was a statistically significant difference in tumor weights of the peptide groups when compared to the control animals (Student's t test p<0.05; see also Figure 15). Kaplan Meier analysis showed a significant survival advantage between peptide mimic groups compared to BSA control group (Figure 16, p=0.015). Furthermore, there was a significant decrease in the percentage of lung metastasis (Figure 17).
The foregoing description of specific embodiments is for the purpose of illustration and is not to be construed as restrictive. From the teachings of the present invention, those skilled in the art will recognize that various modifications and changes may be made without departing from the spirit of the invention.
We claim:

1. A method for prophylaxis and/or therapy of cancer in an individual in need thereof comprising administering to the individual a composition comprising tanned mammalian red blood cells complexed with one or more agents selected from: i) a peptide selected from the group consisting of peptide B1 (HSHKTNLATTPGGG; SEQ ID NO:1), C1 (YPSPVYHSLRSGGG; SEQ ID NO:2), D1 (MHKPWSGHMQVPGGG; SEQ ID NO:3), D2 (HIHGWSPLSSLGGG; SEQ ID NO:4), and combinations thereof; ii) asialoglycophorin; iii) Thomsen-Friedenreich antigen (TF-Ag); and combinations thereof.

2. The method of claim 1, wherein the agent comprises peptide sequence B1 (HSHKTNLATTPGGG; SEQ ID NO:1), C1 (YPSPVYHSLRSGGG; SEQ ID NO:2), D1 (MHKPWSGHMQVPGGG; SEQ ID NO:3), D2 (HIHGWSPLSSLGGG; SEQ ID NO:4).

3. The method of claim 2, wherein the agent comprises C1 (YPSPVYHSLRSGGG; SEQ ID NO:2) or D2 (HIHGWSPLSSLGGG; SEQ ID NO:4).

4. The method of claim 2, wherein the peptide sequence is conjugated to bovine serum albumin (BSA).

5. The method of claim 1, wherein the agent comprises TF-Ag, wherein the TF-Ag is in a complex with BSA.

6. The method of claim 1, wherein the agent comprises asialoglycophorin, wherein the asialoglycophorin is in a complex with BSA.

7. The method of claim 1, wherein the tanned red blood cells in the composition comprise red blood cells obtained from the individual.

8. A composition comprising an isolated population of tanned red blood cells, wherein the tanned red blood cells are complexed with one or more agents selected from: i) a peptide selected
from the group consisting of peptide B1 (HHSHKTNLATTTPGGG; SEQ ID NO:1), C1
(YPSLPVYHSLRSGGG; SEQ ID NO:2), D1 (MHKPWSGHMQVPGGG; SEQ ID NO:3), D2
(HIHGWKSLSSLGGG; SEQ ID NO:4), and combinations thereof; ii) asialoglycophorin; iii)
Thomsen-Friedenreich antigen (TF-Ag); and combinations thereof.

9. The composition of claim 8, wherein the agent comprises peptide sequence B1
(HHSKTNLATTTPGGG; SEQ ID NO:1), C1 (YPSLPVYHSLRSGGG; SEQ ID NO:2), D1
(MHKPWSGHMQVPGGG; SEQ ID NO:3), D2 (HIHGKWSSLSSLGGG; SEQ ID NO:4).

10. The composition of claim 9, wherein the agent comprises C1 (YPSLPVYHSLRSGGG;
SEQ ID NO:2) or D2 (HIHGKWSSLSSLGGG; SEQ ID NO:4).

11. The composition of claim 9, wherein the peptide sequence is part of a conjugated protein,
to bovine serum albumin (BSA).

12. The composition of claim 8, wherein the agent comprises TF-Ag, wherein the TF-Ag is
in a complex with BSA.

13. The composition of claim 8, wherein the agent comprises asialoglycophorin.
IL-2 ELISPOT TF-Ag-BSA Stimulated Mice, IL-2 Producing Cells Per Mouse

- Asialo #268
- C1-BSA #521
- D2-BSA #502
- B1-BSA #577
- TF-Ag-BSA #256
- PBS #552

Mouse

Average IL-2 Producing Cell Number

0 10 20 30 40 50 60 70 80

72
18
14
11
8
3

1/17
Figure 2

IL-2 ELISPOT C1-BSA Stimulated Mice, IL-2 Producing Cells Per Mouse

- C1-BSA #521
- PBS #552

Average IL-2 Producing Cell Number

Mouse

48

9
IL-2 ELISPOT Asialoglycophorin Stimulated Mice, IL-2 Producing Cells Per Mouse

- Asialo #268: 38
- PBS #552: 13

Average IL-2 Producing Cell Number

Mouse
IL-2 ELISPOT Not Stimulated Mice, IL-2 Producing Cells Per Mouse

- Asialo #268
- C1-BSA #521
- B1-BSA #577
- D2-BSA #502
- TF-Ag-BSA #256
- PBS #552

Average IL-2 Producing Cell Number

Mouse
IL-10 ELISPOT TF-Ag-BSA Stimulated Mice, IL-10 Producing Cells Per Mouse

Mouse

Average IL-2 Producing Cell Number

- D2-BSA #502
- Asialo #268
- PBS #552
- C1-BSA #521
- B1-BSA #577
- TF-Ag-BSA #256
Figure 6

IL-10 ELISPOT B1-BSA Stimulated Mice, IL-10 Producing Cells Per Mouse

- B1-BSA #577
- PBS #552
IL-10 ELISPOT Asialo Stimulated Mice, IL-10 Producing Cells Per Mouse

Average IL-10 Producing Cell Number

- Asialo #268: 33
- PBS #552: 112

Mouse
IL-10 ELISPOT Not Stimulated Mice, IL-10 Producing Cells Per Mouse

- D2-BSA #502
- C1-BSA #521
- TF-Ag-BSA #256
- Asialo #268
- B1-BSA #577
- PBS #552

Mouse
Figure 11

Tumor weight versus Vaccine Groups

- Controls
- not significant
- Significant

Tumor weights (mg)

Groups:
- OVA
- BSA
- PBS
- B1
- C1
- D1
- D2
- TF-Ag
- Asialo
Figure 12

**A**

Abs (405 nm) vs Tumor weight (mg)

$R^2 = 0.1422$

**B**

Abs (405 nm) vs Tumor weight (mg)

$R^2 = 0.7752$

**C**

Abs (405 nm) vs Tumor weight (mg)

$R^2 = 0.4669$
Percentage of different numbers of lung metastasis

- controls (15)
- peptides (14)
- carbohydrates (10)

% of lungs

0 1-9 10-24 >25