[Frontiers in Bioscience S4, 840-863, January 1, 2012]

Anti-Thomsen-Friedenreich-Ag (anti-TF-Ag) potential for cancer therapy

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TABLE OF CONTENTS

1. ABSTRACT

Thomsen-Friedenreich antigen (TF-Ag) is the disaccharide (Gal beta1-3 GalNAc alpha), which is also known as the core 1 structure. The presence of this disaccharide on the surface of ~90% of carcinomas is due to altered glycosylation in these tumors. TF-Ag plays a role in the adhesive properties of tumor cells involved in metastasis. Treatment of mice with JAA-F11, a monoclonal antibody to TF-Ag alpha inhibited lung metastasis and improved prognosis in a mouse breast cancer model. The presence of naturally occurring antibodies to TF-Ag in cancer patients is related to improved prognosis. The pancarcinoma expression of TF-Ag, combined with the evidence of a mechanistic role for TF-Ag in cancer spread, show that this target would have clinical utility. The presence of naturally occurring antibody to TF-Ag indicates that increasing the anti-TF-Ag antibody would be safe for the cancer patient and indicates that tolerance would not have to be broken to create this immune response. Finally, the prognostic improvements seen clinically and in animal models indicate that this is an important vaccine target.

2. INTRODUCTION

Thomsen-Friendenreich antigen (TF-Ag) is a cancer vaccine target that has come into its own slowly, with an initial discovery in 1927 by Thomsen (1) followed in 1930 by a more thorough investigation by Friedenreich (2). The initial discovery of TF-Ag was through a phenomenon observed by Thomsen, in which he saw an apparent change in the blood group of red blood cells (rbcs) brought about by standing for 14-25 hours, after which the red blood cells were agglutinated by any human serum. Friedenreich investigated this effect and found that the change was brought about by bacterial enzymes. Friedenreich named the antigen the T antigen after Thomsen, but because of the importance of the information added by Friedenreich, the antigen is usually called the Thomsen-Friedenrich antigen, although occasionally the term "T Ag" is still sometimes used (3). The observation also led to the discovery of neuraminidase as the enzyme responsible for removing the sialic acid which led to the TF-Ag exposure.

Naturally occurring human antibody to TF-Ag was discovered at the same time, as this antibody was the agent which caused the agglutination of the red blood cells

that had TF-Ag exposed on the plasma membrane (1-3). This antibody is present in normal human serum, but not in serum from infants, nor in serum from cord blood. The discovery of this antibody was an early indication that the passive transfer of antibody to TF-Ag and active immunization with anti-TF-Ag would not hurt normal tissue because this naturally occurring antibody did not cause any pathologic effects. The presence of the naturally occurring antibody to TF-Ag was studied by a number of groups. The amount of naturally occurring human antibody to TF-Ag was shown to be of prognostic significance in cancer patients, with a higher amount of antibody related to improved prognosis (4-10). In 1980, Springer also found cell mediated immune response to TF-Ag as measured by a skin test with TF-Ag (11). The TF-Ag used in the skin tests was purified from human blood group O, MN rbcs treated to remove the sialic acid. The purification procedure likely resulted in a preparation of TF-Ag on glycophorin A (12).

The initial discovery of TF-Ag (1) did not show its tumor-association, which was later shown in pivotal work of Springer, with the initial publication in 1974 of TF-Ag expression in breast cancer tissue (13). Springer's wife had died of breast cancer when she was quite young, and Springer became committed to furthering the understanding of cancer, with the ultimate goal of improving prognosis for the cancer patient (11-50).

The use of lectins and antibodies as tools to study TF-Ag led to further understanding of the role of TF-Ag in cancer (13-81). The lectin used for most of these studies is peanut agglutinin (51-61). It must be noted that these reagents have varying specificities, and may bind other structures in addition to TF-Ag. Specificity analyses are important to understanding and will be described later in this review.

The suitability of TF-Ag as an exciting target for cancer immunotherapy has been built slowly, first with the identification of TF-Ag as a tumor antigen (13,14), and then with the identification of TF-Ag in a large percentage of a wide variety of tumors (51-61). About 70% of all known cancers are carcinomas and TF-Ag was found to be a "pancarcinoma antigen" with expression in most of these tumor types. Expression has been shown on cancers of the bladder, breast, colon, liver, ovary, prostate and stomach (1,2,4, 13-61). The production of TF-Ag in these tissues appears to be due to a glycosylation defect, in part due to altered glycosyltransferases in tumor cells compared to the respective normal tissue (4,82,83). Mechanistically TF-Ag is an important target because TF-Ag has a role in the metastasis of tumor cells through its involvement in cell-to-cell adhesion (41,47,48,59,84,85). Since an anti-TF-Ag antibody response could have blocking function, inhibiting metastasis as well as cytolytic function, this antigen could be a very effective target for passive immunotherapy and for a vaccine. In addition, the fact that TF-Ag has a role in the metastatic process decreases the chance that antigen negative cancer cells will develop during immunotherapy.

The following areas are important to the understanding of the importance of TF-Ag as a target in cancer therapy: a) the glycosylation changes that have led to the overexpression of TF-Ag b) the effect of the naturally occurring antibodies to TF-Ag in the cancer patient, c) and d) the specificity and biological effects of the lectins and antibodies that have been used as tools to study TF-Ag in cancer e) the role of TF-Ag in metastasis and f) the progress in the development of a vaccine for TF-Ag. Each of these areas will be covered in separate sections below.

3. SYNTHESIS AND ACCUMULATION

TF-Ag (Gal beta1-3GalNAc alpha-Ser/Thr) is categorized with the mucin-associated epitopes. The disaccharide structure of TF-Ag is the core 1 structure of O-linked mucin type glycans. On various normal cell membranes this disaccharide is hidden either by tertiary structures or by highly negatively-charged sialic acid, preventing TF-Ag from exposure to the immune system, but in tumor cells this structure is exposed. The surface expression of TF-Ag on tumor cells could be due to increased synthesis of the core 1 structure, decreased synthesis of enzymes which would lead to the production of more complex carbohydrates or increased synthesis of glycosidases which would transform more complex structures to this simple core 1 structure (Figure 1)(4, 23, 86-92).

The TF-Ag structure is synthesized by addition of galactose from UDP-galactose to GalNAc alpha-Ser/Thr (Tn antigen) catalyzed by core 1 beta1,3 Gal-transferase (T synthase). TF-Ag can be modified to become the core 2 structure Gal beta1-3 (GlcNAc beta1-6) GalNAc alpha-Ser/Thr which can be further modified into complex O-glycans in normal epithelium. TF-Ag can also be modified by sialyltransferases to become either the monosialylated Neu5Ac alpha2-3 Gal beta1-3 GalNAc alpha-Ser/Thr or Gal beta1-3 (Neu5Ac alpha2-6) GalNAc alpha-Ser/Thr, or the disialylated Neu5Ac alpha2-3 Gal beta1-3 (Neu5Ac alpha2-6) GalNAc alpha-Ser/Thr (86,92). TF-Ag can also be modified by N-acetylglucosamine addition to the Gal, or by fucosylation or sulfation (86,92).

Decrease in any or all of the enzymes that add to Core 1 could result in the increase in the expression of TF-Ag in cancer tissue. In unpublished data we have characterized a variant of the 4T1 breast tumor cell line which does not metastasize and have found increased 2-3 sialyltransferase activity and decreased expression of TF-Ag on its surface. This supports the idea that decreases in enzymes which add complexity to the Core 1 structure may be involved in the upregulation of TF-Ag in tumor cells.

T-synthase is the only enzyme which forms core 1 from the Tn antigen precursor. T-synthase transfers Gal from UDP-Gal to GalNAc alpha- Ser/Thr (4, 86-92). There is no difference in the amount of this enzyme in normal and tumor tissues so variability in T-synthase is not related to TF-Ag expression. T-synthase requires an ER-localized molecular chaperone called Cosmc (Core 1 beta1,3 Gal-T-specific molecular chaperone) to function. Cosmc specifically promotes folding/stability of the core 1 beta1,3

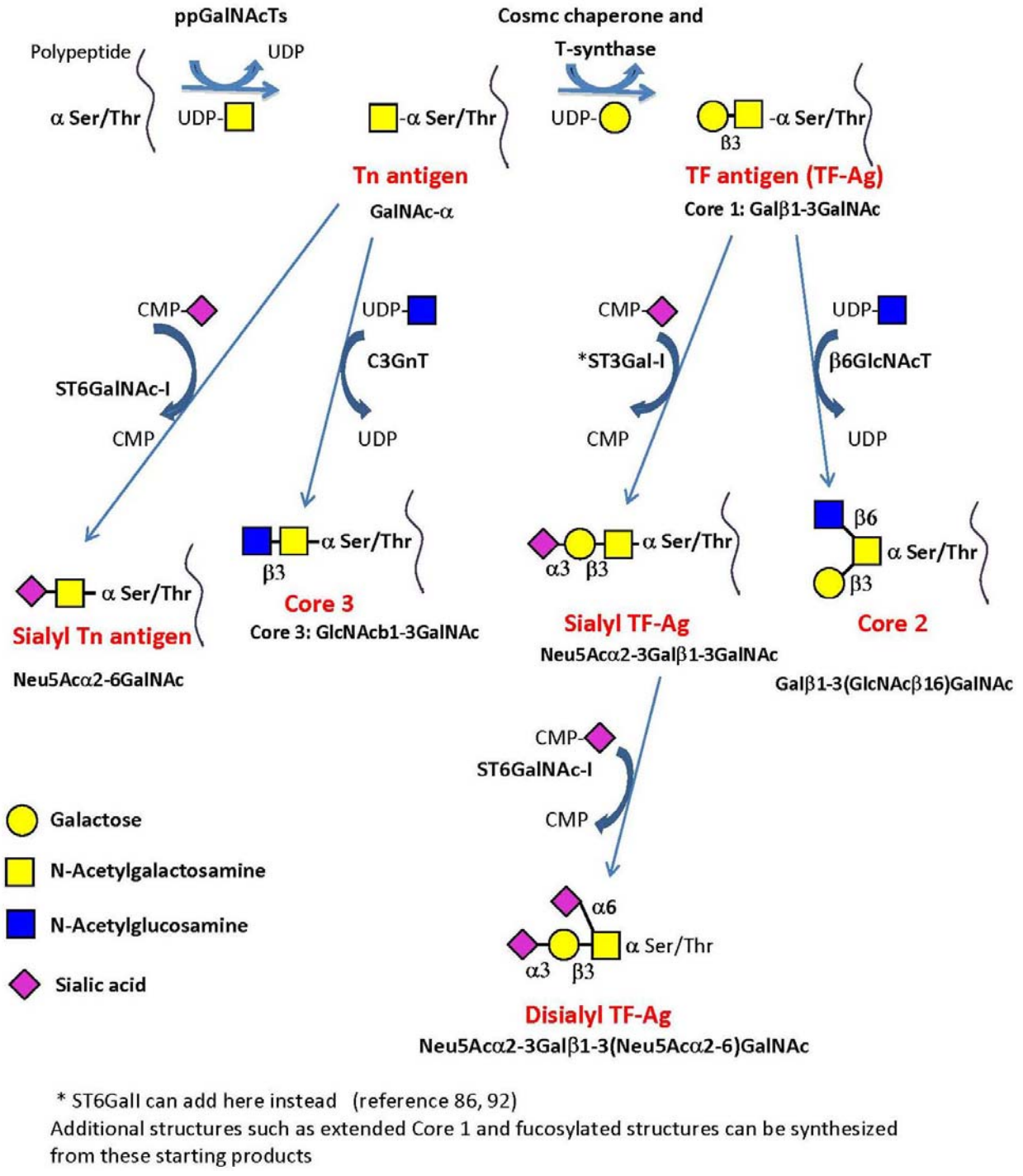

Figure 1. The synthetic steps involved in Thomsen-Friedenreich antigen formation and in masking of Thomsen-Friedenreich antigen.

Gal-transferase (86,89, 92). Alteration in levels of Cosmc would affect the amount of TF-Ag. In addition, increased availability of the nucleotide sugar substrate UDP-galactose for the T-synthase could lead to increased TF-Ag on tumor cells and increased amount of UDP-Gal are seen in tumor cells (4,89,92). However, increased amounts of the Tn Ag precursor would also be required.

Tn synthesis utilizes polypeptide alpha- N-acetylgalactosaminyltransferases which transfer UDP-GalNAc to serine or threonine on a protein. Enzymes which

perform this function are called ppGalNAcTs. More than one enzyme has this activity, and in humans 24 of these genes are present. Slight differences in the preference for amino acid sequence exist for these enzymes, but most exhibit a preference for this first step of glycosylation for a serine or threonine which has a proline at +3, -3, +1 or -1 from it (86,94). These transferases have a lectin domain and due to this domain they more efficiently add subsequent glycosylation to the protein after the first addition. This would help create the heavily glycosylated mucins that express TF-Ag such as MUC-1 and CD44. These enzymes are in the Golgi apparatus and act on folded proteins that have exited the endoplasmic reticulum (86,92, 93, 149). Polymorphisms in MUC-1 amino acid structure which lead to large tandem repeats are associated with increased TF-Ag expression. This may be due to a locally high concentration of Tn for action by T-synthase, or a binding area for the T-synthase (91, 149).

Tn is a precursor for two other structures in addition to TF-Ag. Tn becomes Core 3 with the action of Core 3 beta1-3 N-acetylglucosaminyltransferase, and this reaction requires a UDP-N-acetylglucosamine. Core 3 is subject to further modification. Tn can also become Sialyl-Tn with the action of the sialyltransferase ST6GalNac-I and cytidine monophosphate N-acetyl neuraminic acid. Decreases in the availability of these enzymes and substrates can cause an increase the amount of Tn that can be converted to TF-Ag (86,92). The synthetic steps involved in Thomsen-Friedenreich antigen formation and in masking of Thomsen-Friedenreich antigen are summarized in Figure 1.

Finally, TF-Ag production may be increased because TF-Ag production is increased when the Golgi is more alkaline. It has been shown that the medial/trans-Golgi pH (pH$\geq$ 6.75) of human breast and colon cancer cells is generally higher than that in non-cancerous control cells (pH 5.9-6.5) (90). A differential increase in pH of 0.2 was shown to increase the amount of TF-Ag produced, suggesting that a decrease in pH may also suppress surface TF-Ag levels in non-cancerous tissue.

Several studies have been performed to determine which human glycoproteins carry the TF-Ag epitope. High molecular weight mucins appear to be the likely candidates, but this may vary among different types of cancers. A strong association has been found with the mucins MUC-1 and 2 and TF-Ag in colon, breast, and other adenocarcinomas (52,53,67-69). Mucins are known to be highly O-glycosylated, and the most abundant glycan moiety has a core 1 or 2 basis, so it would be easy to explain how this TF-Ag is expressed following aberrant glycosylation or glycosidase saccharide removal.

Aberrant glycosylation can be the result of more than one mechanism, and indeed four mechanisms have already been suggested. In the study described above a MUC-1 VNTR polymorphism was found to be associated with increased TF-Ag expression (91). Another study described above found that increasing the pH of the Golgi, increased TF-Ag expression (90). In addition, on B cells transient surface TF-Ag expression can occur due to surface sialidases (87) and our recent study has shown decreased sialyltransferase mRNA expression levels associated with metastatic cell lines. The first alteration would cause a MUC-1 specific increase in TF-Ag expression while other glycoproteins would not have an upregulation of TF-Ag. The latter 3 mechanisms would create a general increase in TF-Ag expression in a wide variety of glycoproteins. Support for the latter 3 of these mechanisms has been shown in other studies which identify a second protein with TF-Ag association, the high molecular weight variants of CD44 found in colon cancer as the carrier of TF-Ag epitopes. CD44 is known to be involved in cell-cell adhesion, and the expression of TF-Ag and potentially other altered glycosylation products may contribute to the organ-specific metastasis seen in colon cancer (104,149).

4. LECTINS

Much of the early work concerning TF-Ag was done with lectins as tools and work continues with these reagents so it is important to understand the uses and limitations of these molecules. Lectins are multivalent, nonenzymatic carbohydrate binding proteins. Owing to their specificity in sugar binding they are used as probes in many biological systems (94, 95). They are used as biomarkers to differentiate between normal and malignant cells and are also used in purification and characterization of glycoconjugates (96).

The table below gives an overview of some ligands with respect to their TF-Ag anomer specificity, substitutions accepted by the lectin and effect of lectins on the proliferation of cancer cells. TF-Ag anomeric specificity refers to whether the reagent binds Gal beta1-3GalNAc alpha (TF-Ag alpha) and Gal beta 1-3GalNAc beta (TF-Ag beta) or binds TF-Ag alpha alone.

The following are descriptions of some of the lectins with reactivity with TF-Ag. A matter of concern when contemplating targeting TF-Ag is that some lectins and antibodies which bind to TF-Ag cause slight tumor cell proliferation (lectins- a maximum of 46% +/- 4%)(60) (antibodies- a maximum of 31% +/- 8%)(80) while other antibodies and lectins which bind TF-Ag cause slight tumor cell growth inhibition (lectin- a maximum of 43% +/- 12)(antibodies a maximum of 36.5%) (60) These effects must be fully understood before undertaking vaccine development. The lectins will be described in two classes-cell proliferation stimulating lectins and cell proliferation inhibiting lectins.

4.1 .Proliferation stimulating lectins-

4.1.1Mechanisms of cell proliferation

Cancer cell proliferation may be caused by aggregation on the cell surface with ligands that are involved in phosphorylation and ultimately proliferation. However, internalization of one of the monoclonal antibodies (TF5) that had this effect seemed necessary for its effects. These mechanisms are not well understood.

4.1.2. Peanut agglutinin- (PNA)

Peanut agglutinin (*Arachis hypogaea*) is one of the most important lectins used in the detection of malignant cells because of its long use for the detection of the TF-Ag (94). PNA has higher binding specificity for the beta anomer of TF-Ag than the alpha anomer. It mainly recognizes Gal and its derivatives at the nonreducing end of the sugar and its binding site has maximum complementarity with asialo GM1 sequence (97, 98). Therefore PNA can react with normal healthy cells expressing asialoGM1 ganglioside. It may thus react with spleen- white and red pulp (99), kidney tubules (100,101), regenerating respiratory epithelial cells (100) and natural killer (NK) cells (102,103). NK cells play a major role in mediating antitumor effect of radiation therapy. If used clinically, PNA binding of NK cells may lead to the depletion of these NK cells thus interfering with its antitumor effect. PNA's asialo GM1 binding is thus of utmost concern (103). Apart from TF-Ag and asialo GM1, it can also bind to lactose, alpha/beta methyl Gal and lacto-N-biose (Gal beta1-3 GlcNAc) (98).

The specificity of PNA for TF-Ag is attributed to the hydrogen bond mediated interaction between the carbonyl oxygen atom of acetamido group of the second hexopyranose ring of TF-Ag and Asn-41 side chain of PNA. PNA strongly binds the nonsialylated form of TF-Ag. Steric hindrance and charge of sialic acid group are considered to be the factors preventing the lectin-ligand interactions. (94,97). TF-Ag is nonsialylated on cancer cells while on normal cells it is present in sialylated form, less interaction of nonsialylated TF-Ag with PNA is thus advantageous in cancer detection studies.

PNA has stimulatory effect on cell proliferation which might cause cancer progression (98). The stimulatory effect of PNA does not require internalization. Studies with radiolabeled PNA showed that at 24 hours the localization index for I125 labeled PNA was 8.6 for tumors and 8.7 for kidneys; and even after 72 hours there was higher localization of the labeled PNA in kidneys than in tumors (104). This is likely due to the asialoGM1 ganglioside in the kidney tubules and the preference of PNA for the beta linked GalNAc. Due to the above mentioned factors PNA cannot be used as an effective cancer detection or therapeutic tool.

4.1.3. *Amaranthus caudatus* lectin (ACL or ACA)

The specific sequence of binding of ACL with TF-Ag is strongest with the GalNAc at the reducing end as shown by the ability of this monosaccharide to inhibit the lectin, whereas Gal alone could not inhibit. However, Gal beta 1-3GalNAc beta1-Me inhibits slightly better than Gal beta 1-3GalNAc alpha1-Me indicating the ability of this lectin to bind both the alpha and beta anomeric configurations (96,97, 105). Some articles suggest that this is binds alpha configurations only but the data cited above from Wu *et al* disagrees with this conclusion (96,97, 105). Although ACL has low affinity for human blood group precursors (Gal beta1-3/4GlcNAc beta), it binds to their polyvalent forms. ACL does not react with Gal or its derivatives (97). The efficient binding between ACL and its ligand requires free hydroxyl groups at C4 of both Gal and GalNAc and N-acetyl group on C-2 of GalNAc (106). Hence ACL does not react with lacto-N-biose (Gal beta 1-3 GlcNAc).

ACL has shown dose dependent stimulatory effect on the cell proliferation in case of colon cancer. 20 micrograms/ml of ACA increased the cell number of HT29 (human colon cancer cell line) by 27+/-12%. It also binds to sialylated as well as non sialylated TF-Ag with equal affinity (106). Therefore it cannot be used to differentiate between normal and cancerous cells. Binding studies with HNPCC (hereditary nonpolyposis colorectal cancer) and FAP (Familial adenomatous polyposis) cell lines have suggested that ACL can be used to identify the zones of abnormal proliferation in case of FAP. FAP is a genetic disease in which failure of control of cell proliferation lead to the formation of adenomatous polyps which may then lead to colonic cancer. It can also be used as a histochemical reagent for the biopsy studies of HNPCC (106).

4.2. Proliferation inhibiting lectins-

4.2.1. Mechanisms of cell proliferation inhibition-

The lectins which inhibit cell proliferation have no cytotoxic effects on the cells which indicates that inhibition of cell division is the mechanism of action of decreased cell numbers after incubation with these lectins. Protein exchange between the cytoplasm and nucleus through the nuclear pores is essential for cell growth and division. There are at least three mechanisms for the transport of proteins into the nucleus. One of them is dependent on the Nuclear localization sequence (NLS). In this pathway, the proteins bind to ligands which then form a complex with the NLS receptors. The movement of the ligand-NLS receptor complex transports the proteins into a nucleus. ABL internalization is required and it has been found to inhibit the NLS dependent pathway of protein uptake. The exact mechanism is yet to be discovered (107).

A different inhibitory mechanism was suggested for jacalin. It was concluded that jacalin induces phosphorylation of putative HLA class II-associated protein I. This causes release of protein phosphatase 2A from a PP2A-PHAP1 complex. PP2A is activated as it is released and this induces dephosphorylation and subsequent deactivation of extracellular-signal-regulated kinase (ERK) (108).

4.2.2. Jacalin

Jacalin is obtained from *Artocarpus heterophyllus*. It binds to Gal beta1-3 GalNAc alpha Me more strongly than Gal beta1-3 GalNAc **beta** Me (109). Thus, jacalin has a significantly higher affinity for the TF-Ag alpha anomer than the TF-Ag beta anomer (110). Moreover, it has higher affinity for Gal beta1-3 GalNAc alpha-Ser than Gal beta 1-3 GalNAc (109). It does not bind to A, B blood group antigens nor to asialo GM1 (109). Thus jacalin is highly specific for TF-Ag alpha anomer that is the tumor antigen. However, it binds to both sialylated and nonsialylated forms of TF-Ag, but binds more strongly to the asialylated form found in tumors (110, 111). The C-

4 and C-6 of GalNAc are important for the lectin-ligand interaction (105,112) and jacalin does not bind to lactose and N-acetyl lactosamine. It binds to many glycoproteins containing the glycan Gal beta1-3 GalNAc alpha-O-. These include human IgA1, bovine coagulation factor X, bovine protein Z plasminogen and chorionic gonadotropin (110).

Jacalin has been used as a histochemical tool to differentiate between normal and malignant cells (110, 113,114). It has also been used to examine the development of cervical intraepithelial neoplasia (110, 119). Jacalin's binding affinity for IgA has been utilized for isolation of IgA1 from human serum as well as for IgA nephropathy investigation (110). Jacalin has a non-cytotoxic, dose dependent inhibitory effect on cell proliferation. It has been observed that Jacalin (20micrograms/ml) reduces the cell number of HT29 (human colon cancer cell line) by 43+/-12% (105). However, it has been speculated that owing to its erythrocyte agglutination property and mitogenicity for CD4+T cells, jacalin will have toxic effects if administered systematically (116).

4.2.3. *Agaricus bisporus* lectin (ABL)

Agaricus bisporus lectin binds strongly to subterminal GalNAc of the alpha anomer of TF-Ag (98). Gal beta1-3 GalNAc and Gal beta1-3 GalNAc alpha are the most strongly bound disaccharides while lactose and Gal beta1-6 GlcNAc also bind to ABL. ABL's poor affinity for Gal and GlcNAc indicates the importance of axial C-4 hydroxyl group in GalNAc and C-2 acetamido addition to Gal (117). ABL reacts with nonsialylated as well as sialylated forms of TF-Ag (108). It shows reversible anti-proliferative activity (60). ABL's effect was tested in different cell lines. It was observed that ABL caused 87% inhibition of thymidine incorporation in HT29 colon cancer cells, 16% inhibition in Caco-2 (another colon cancer cell line), 50% in MCF-7 breast cancer cells and 55% in Rama-27, rat mammary fibroblasts (108).

4.2.4. *Sclerotium rolfsii* lectin (SRL)

SRL has much higher reactivity for O-linked sugars when compared to N-linked sugars (118). It binds with the ligand GalNAc of alpha anomer of TF-Ag. Apart from core 1, it also reacts with core 2 (Gal beta1-3 (GlcNAc beta1-6) GalNAc alpha) and core 8 (Gal alpha1-3 GalNAc alpha). SRL does not bind to asialo-GM1 and hence does not react with NK cells (118, 111).

The C4-OH group GalNAc and C2-OH group of Gal are very essential for the interaction between SRL and TF-Ag. Substitutions at C3 position with sulfates, sialic acid and N-acetylglucosamine increase the binding affinity (118). N-acetyl group of TF-Ag is involved in the binding interaction with the primary as well as secondary binding site of SRL. GalNAc interacts with the primary site whereas GlcNAc reacts with the secondary binding site (119).

4.2.5 *Agrocybe aegerita* lectin (AAL)

Crystal structure analysis of the AAL-TF-Ag complex has revealed many residues of AAL and TF-Ag that are involved in the binding interaction. AAL has high affinity for sulfated TF disaccharide. The two residues of AAL that play a major role in binding are Arg85 and Glu66. Arg 85 is responsible for binding affinity and it binds to the O5 of the galactoside ring in GalNAc of TF-Ag while Glu66 which is important in determining the specificity, binds to N-acetyl group. These interactions are mediated through water molecules. It has been observed that the water molecules on the surface of carbohydrate recognition domain contribute to increase the affinity and specificity of a lectin towards the glycan. AAL binds to the alpha anomer of TF-Ag and the chair conformation of the antigen is required for stability and recognition specificity of the AAL-TF-Ag complex. (120).

4.2.6 *Beauveria bassiana* lectin (BBL)

The alpha anomer of TF-Ag is the potent ligand for *Beauveria bassiana* lectin. BBL reacts effectively with the sialylated derivatives of TF-Ag to cause hemagglutination of human type O red blood cells. It has a very low affinity for the beta anomer. It does not bind to single sugar residue glycoconjugates. Although it binds to both the forms, binding with the sialylated form is much weaker than the nonsialylated ones. The binding profile of BBL is Gal beta1-3 GalNAc > Neu5Ac alpha2-3 Gal beta1-3 (Neu5Ac alpha2-6) GalNAc > Gal beta1-4 Glc alpha. The lectin is not very specific for O-glycoproteins and also binds to asialo Tamm-Horsfall glycoprotein (N-glycoprotein) (95).

4.2.7 *Xeocomus chrysenteron* lectin (XCL)-

XCL recognizes galactose and N-acetylgalactosamine. (108). It binds more strongly with the nonsialylated form of TF-Ag. It has been observed that the affinity decreases in the presence of sialic acid (108), while it increases if a galactose is attached to N-acetylgalactosamine. XCL shows antiproliferative effect as well as insecticidal activity (121).

In summary, the study of lectins and their binding interactions with TF-Ag can direct the research on selecting a suitable antibody with high binding affinity and anti-proliferative effect. It has been concluded that the lectins binding to both the beta and alpha anomer usually stimulate the proliferation of cancer cells while those binding to the alpha anomer inhibit proliferation (98). Irazoqui (98) also suggests that stimulation or inhibition of proliferation is due to which side of TF-Ag is bound to the molecule. These conclusions were based on a comparative study of ABL and PNA, and monoclonal anti-TF-Ag antibodies. Irazoqui (98) found that lectins which have high Gal beta1-3 GalNAc alpha1-4 GalNAc beta1-4 GlcNAc beta1-3 Man beta1-4 Glc beta-Cer, low Gal beta1-3 GalNAc beta1-4 GlcNAc beta1-3 Man beta1-4 Glc beta-Cer and no Ga1(Gal beta1-3 GalNAc beta1-4 Gal beta1-4 Glc beta-Cer) reactivity have tumor specificity and will also have inhibitory effect on cell proliferation (98). In other related work, Yu et al (105) suggest that the differing proliferative effects are due to the different specificity for sialylated or non-sialylated structures or the different affinities for clustered TF-Ag. From the

Table 1. Specificities of lectins used in TF-Ag research

Lectin: those with known tumor specificity are bolded	**TF-Ag anomer specificity**	**Substitution accepted**	**Effect on cell proliferation**
Peanut agglutinin (94,97-104) PNA	Higher for beta anomer	Nonsialylated TF-Ag, asialo GM1, lacto-N-biose, lactose, Gal and its derivatives, core 2	Proliferative
Amaranthus caudatus lectin (96,97,105,106) ACA	Alpha and beta anomer	sialylated and nonsialylated, core 2	Proliferative
Jacalin (105, 109-116) JAC	Alpha anomer	sialylated TF-Ag, core 3	Inhibitory
Agaricus bisporus lectin (98,108,117) ABL	Alpha anomer	sialylated and nonsialylated, Gal beta1-3 GalNAc alpha Lactose, Gal beta1-6 GlcNAc, core2	Inhibitory
Sclerotium rolfsii lectin (111,118,119) SRL	Alpha anomer	Sialylated TF-Ag, core2, core 8	NA
Agrocybe aegerita lectin (120) AAA	Alpha anomer	Sulfated TF-Ag	Inhibitory
Beauveria bassiana mycelium lectin (95)	Alpha anomer	Nonsialylated, asialo tamm-Horsfall glycoprotein	NA
Xerocomus chrysenteron lectin (119,121) XCL	Alpha anomer	Nonsialylated	Inhibitory

TF-Ag alpha - Gal beta1-3 GalNAc alpha, TF-Ag beta- - Gal beta1-3 GalNAc beta, Core 2- Gal beta1-3 (GlcNAc beta1-6) GalNAc alpha, Core3- GlcNAc beta1-3 GalNAc alpha, Core8- Gal alpha1-3GalNAc alpha, Sialylated TF can be any of the following, Neu5Ac alpha2-3 Gal beta1-3 GalNAc alpha, Gal beta1-3 (Neu5Ac alpha2-6) GalNAc alpha, Neu5Ac alpha2-3 Gal beta1-3 (Neu5Ac alpha2-6) GalNAc alpha

summary in Table 1, it appears that lectins which bind alpha and beta derivatives of TF-Ag cause a proliferative effect whereas lectins which cause inhibition of proliferation bind to TF-Ag alpha linked and **not** beta linked. Further research, including molecular modeling will be required to understand these effects.

5. MONOCLONAL ANTIBODIES

Attempts at producing monoclonal antibodies (mAb) to TF-Ag have been made using neuramindase-treated rbcs (77), TF-Ag positive lung tumor cells (125), and synthetic TF-Ag compounds (126, 79) as antigens. These vaccinations have mostly produced specific IgM antibodies which because of their large molecular weight and lower affinity have limited clinical utility. IgM antibodies may be useful in immunohistochemistry but not *in vivo* in the cancer patient.

Antibodies are more specific than lectins but antibodies to TF-Ag can either bind both the alpha and beta linked TF-Ag or bind to just the tumor associated TF-Ag alpha. Antibodies to TF-Ag, like lectins to TF-Ag can cause *in vitro* cancer cell growth enhancement or retardation. Irazoqui *et al* (98), also showed that antibodies which stimulated proliferation bound mainly to the terminal Gal, while antibodies which inhibited proliferation bound mainly the GalNAc of an alpha linked TF-Ag. This is similar to the reactivity shown with lectins. Dahlenborg (62) created 3 human monoclonal antibodies to TF-Ag which were studied further by Irazoqui (98) and Yu (80). In Dahlenborg (62) the antibodies TF1 (IgM), TF2 (IgA) and TF5 were listed as TF-Ag **beta** specific, with TF5 (IgM) also reacting with Gal beta1-3 GlcNAc. The binding of TF2 and TF5 to TF-Ag was also inhibited by high concentrations of galactose while TF1 was not. In their hands nothing was inhibited by their synthetic TF-Ag alpha linked structure but the reactivity with neuraminidase treated rbcs and asialoglycophorin would lead one to question the integrity of their synthetic compound and this lack of reactivity. The lack of inhibition of TF1 by galactose in comparison to TF2 and TF5 would indicate a more GalNAc reactivity of this antibody. Yu *et al* (80) performed cancer cell proliferation studies with these antibodies and TF1 did not cause cancer cell proliferation in HT29 human colon cancer cells although TF2 and TF5 did. None of the antibodies caused stimulation of Caco-2 cells, while HT29-MTX had increased proliferation (~10% with TF1, ~15% TF2, ~18% TF5) and LS174T cells showed enhanced proliferation with TF1 and TF5 (~10% with TF1, ~20% with TF5). Irazoqui performed inhibition assays with these human monoclonal antibodies and found that PNA and TF2 and TF5 were similar in specificity, with a preference for the beta Gal whereas binding of TF1 to asialoglycophorin was only inhibited by TF-Ag alpha compounds. The conclusions of Irazquoi about both monoclonal antibody and lectins and proliferation are given in the lectin section (above), with the side of the antigen that is bound by the antibody playing a dominant role. Two of these human monoclonal antibodies to TF-Ag reacted with both the alpha and beta structures, and one reacted with TF-Ag alpha only. It is not clear if this is representative of what is seen in the patient. It would be of clinical interest to determine if the increased levels of anti-TF-Ag in cancer patients that are associated with prognosis are TF-Ag alpha specific or if it reacts with both the alpha and beta linked TF-Ag structures. If the antibody in patients that is related to improved prognosis is TF-Ag alpha specific then it is important in vaccine efforts to make this limited specificity response. If the antibody in these patients reacts to both the alpha and beta derivatives it is possible that *in vitro* cancer cell proliferation is not related to *in vivo* effects, and limitation of the reactivity of the antibody in a vaccine response would not be necessary.

Longenecker *et al*. produced an anti-TF Ab (170H.82) through immunization with a synthetic TF-Ag linked to HSA (76). No mouse radiolocalization testing of 170H.82 antibody has been reported, but the Longenecker group did perform *in vivo* tumor localization experiments with 2 other similar reagents which also bind both the TF-Ag-alpha and beta derivatives: radiolabeled peanut lectin (PNA) (51) and radiolabeled mAb 155H.7 (74) which they reported to be similar to mAb 170H.8 (76). In their mouse experiments with the 155H.7 antibody which has a specificity similar to 170H.82, the kidney to tumor ratio was 0.75, and the kidney was higher than any organ except the thyroid (pretreatment with cold iodine was not

performed) (76). The m170H.82 was tested in humans as an immunolocalization reagent (Tru-Scint ^{TM}) and seemed promising, even though it binds both the alpha and beta anomers of TF-Ag, showing an axillary nodal involvement sensitivity of 71% (5/7), and specificity of 89% (8/9) (51,76). This specificity was calculated after they disregarded the kidney staining seen in most patients (76). This monoclonal antibody reappeared as m170 in radioimmunotherapy for treatment of metastatic prostate cancer (126). In this radioimmunotherapy with ^{90}Y labeled m170, tumor in bone and in lymph node received between 10 and 11 Gy/GBq, while liver received 3.95 and kidney received 2 Gy/GBq. Thirteen of the 17 patients treated had bone pain, and of these 5 had complete resolution of pain and 2 had 50% reduction. Some moderation of pain lasted a mean of 4.3 weeks.

Although it is clear that the tumor associated TF-Ag is Gal beta1-3 GalNAc-alpha-O (alpha-linked), many of the antibodies and other reagents used to study and identify this antigen have bound to both the tumor associated Gal beta1-3 GalNAc-alpha-O and the non-tumor associated Gal beta1-3 GalNAc-beta-O. Antibodies and other reagents which bind to both alpha and beta anomers showed fairly good tumor specificity, however, they are limited by their binding *in vivo* to normal kidney, and some potential binding in the central nervous system to GM1 ganglioside, the asialo-GM1 of NK cells (98,117), or the GD1 of glycolipids. Even while binding to the alpha and beta anomers, Tru-Scint showed a respectable 83% sensitivity and a 97.7% positive predictive value in the detection of metastasis (77).

In our laboratory, a mouse monoclonal IgG_3 antibody, JAA-F11, has been produced which specifically binds to tumor associated TF-Ag alpha and not TF-Ag beta (79,85, 98,129,133,140). Using an array composed of 442 different carbohydrate structures, JAA-F11 mAb was shown to be very specific for TF-Ag. The specificity of JAA-F11, with lack of binding with the beta-linked asialo-GM1 of normal kidney tubules, should eliminate the kidney binding of the previous, less specific, anti-TF-Ag antibody (170H.82) that showed some success in targeting tumor tissue but bound to normal kidney. In addition, JAA-F11's lack of reactivity with this structure is important for passive immunotherapy because this structure is on NK cells and NK cell depletion has been shown to result in accelerated tumor growth which attenuates the anti-tumor effects of irradiation (98,117). JAA-F11 also did not react with structures with additions on C-3 or the C-2 of the galactose indicating that extensions in these areas on extended Core 1 or Core 2 structures would block binding of JAA-F11 to other non-tumor tissues. JAA-F11 causes a slight but significant decrease in mouse and human cancer cell growth.

The glycan analysis is consistent with the tumor specificity of JAA-F11 mAb seen in *in vivo* imaging with both mouse 4T1 breast tumor in a mouse model and the human MDA-MB-231 breast tumor in a SCID mouse. MDA-MB-231 is a *triple negative tumor *(estrogen receptor, progesterone receptor and Her2 negative). Successful imaging of the human triple negative breast cancer cells supports the idea that JAA-F11 will provide better imaging and therapeutic results than other anti-TF-Ag antibodies. Targeting triple negative breast tumors is significant because these tumors are more difficult to treat, are often more aggressive and often occur in younger women. This is an indication that a TF-Ag vaccine would be useful for this group of patients along with patients with other carcinomas.

The ability of JAA-F11 to block tumor metastasis was shown *in vivo* in a 4T1 breast cancer mouse model, and *in vitro* and *ex vivo* for human tumor cell lines. In the mouse model, JAA-F11 blocked lung metastasis of 4T1 breast tumor cells by blocking adhesion of the metastasizing cancer cells to the vascular endothelium. A significant decrease in the number of metastatic foci in the lungs of JAA-F11 treated animals was seen ($p=0.016$), with > 50% of the treated animals metastasis free (140). JAA-F11 has also been shown to inhibit cancer cell adhesion to cells of the blood vessels in two *in vitro* models of human cancer cell metastasis. Inhibition of binding of human tumor cells to human umbilical vein endothelial cells and to human bone marrow endothelial cells is shown with JAA-F11 treatment (140, 146,147). JAA-F11 treatment also blocks metastasis in an *ex vivo* model of metastasis in which tumor cells flowing over porcine dura mater stick and roll, an interaction which is blocked by JAA-F11 antibody but not by control antibody. Gassman *et al* in 2009(85) found that JAA-F11 could also block human HT-29LMM and T84 colon cancer cells from binding to lung microvasculature via a unique quantitative *in vivo* microscopic analysis. This will be described in more detail in the metastasis section.

In summary JAA-F11, an antibody specific for the TF-Ag alpha linkage inhibits growth of human and mouse tumor cells in vitro, causes a survival time extension in mice bearing the 4T1 metastatic breast tumor and causes >50% decrease in lung metastasis in the 4T1 mouse model. MicroPET imaging in mice has shown that JAA-F11 localizes to TF-Ag positive mouse and human breast tumors *in vivo*. In addition, JAA-F11 inhibits adhesion of human breast carcinoma cells to blood vessels *in vitro, ex vivo* and *in vivo*. Importantly, MicroPET imaging in mice has also shown that JAA-F11 localizes a triple negative human breast cancer cell line indicating that JAA-F11 therapy could be useful in this group that is difficult to treat (79,85,98,129,133,140). JAA-F11 has also been shown to be effective in experiments demonstrating inhibition of breast, prostate and colon tumor metastasis (79,85,98,129,133,140). Collectively, these results indicate that JAA-F11 has considerable potential for radiolocalization and for passive immunotherapy of cancer.

The inhibition of metastasis and improvement of survival with passive transfer with JAA-F11 is an indication that a vaccine would create protective effects in the cancer patient. It is important to determine if the vaccine must cause development of a TF-Ag alpha response or if a TF-Ag alpha and beta response is appropriate. The experiments suggested above to define

the specificity of human anti-TF-Ag related to prognosis in treated cancer patients is part of this answer. Additional experiments with the same mouse model could to determine, if a mouse monoclonal antibody, which unlike JAA-F11, bound to both TF alpha and beta blocked metastasis and improved survival. In addition, an antibody which stimulated *in vitro* tumor cell growth should be selected to determine if these *in vitro* cancer cell proliferation assays have any relevance to the clinical situation *in vivo*.

6. HUMAN IMMUNITY TO TF-AG

6.1. Human anti-TF-Ag antibodies

Natural anti-TF-Ag antibodies are found in the blood of every individual (127), but are significantly decreased in individuals bearing TF-Ag-positive cancers and pre-malignant conditions (128), although there are slight variations. Levels of anti-TF-Ag antibodies in healthy people are constant, and are persistent throughout life after infancy (127). These antibodies are believed to be elicited primarily by the intestinal flora (50). Bacterial TF-Ag-expressing molecules are thought to be largely responsible for the anti-TF-Ag antibodies that humans naturally possess and this is supported by the fact that persons depleted of their intestinal flora by oral antibiotics have little circulating anti-TF-Ag Ab (50).

Georg Springer and colleagues were the first to elucidate the carcinoma association of TF-Ag with their finding that breast cancer tissue expressed TF-Ag and that the patient had antibody to this tumor antigen (13). Expression of the TF epitope on human carcinomas is usually accompanied by a decrease in the anti-TF-Ag antibodies in the serum (129). In studies done by Desai and colleagues, a semiquantitative standard hemagglutinin titration was used to determine the anti-TF-Ag levels of carcinoma and control patients (50). In serum pools of healthy humans, the titer score ranged from 20-25 (50). It was discovered that 36.6% of preoperative patients with carcinomas had severely depressed anti-TF-Ag hemagglutination scores (≤12) whereas 4.2% had highly elevated anti-TF-Ag scores (≥44); healthy individuals' anti-TF-Ag scores were depressed in only 8.5% of healthy persons and 9.4% of individuals with benign disease (13,50). The hemagglutination assay performed likely measured IgM anti-TF-Ag. They also found that curative surgery to remove the breast carcinoma in individuals resulted in a substantial increase (>25%) in anti-TF-Ag antibody titer scores 1 to 5 months later in 73.8% of breast carcinoma patients and 100% of lung carcinoma patients studied (50). This highly significant increase in anti-TF-Ag of the majority of patients whose carcinoma had been removed shows that the TF-Ag in carcinoma may react with the anti-TF-Ag antibodies removing some from the serum.

Since it is established that carcinoma patients have humoral responses to the TF-Ag, it is of importance to determine the types of antibodies involved in this response. Desai and colleagues revealed that affinity purified polyclonal anti-TF-Ag antibodies from a normal human serum pool contained 64% IgM, 32% IgG, and 4% IgA (39). This showed that the anti-TF-Ag antibodies are predominantly IgM. In studies done by Oleg Kurtenkov and colleagues, the levels of anti-TF-Ag antibodies in patients with gastric cancer were lower than in healthy controls irrespective of stage of disease (130). The amount of anti-TF-Ag specific IgG antibody level in patients does not change as much as the IgM anti-TF-Ag antibodies. The IgM anti-TF-Ag antibody has been found to show dramatic changes, uniformly falling by the early stages of cancer (39,129). Low levels of these antibodies were also shown to be associated with pre-malignant conditions and with a higher risk of cancer development (130, 34). Springer also demonstrated that carcinoma patients but not healthy people, showed cellular immunity to TF-antigen both *in vivo* and *in vitro* (50). Cellular immunity was shown *in vivo* by delayed-type skin hypersensitivity (DTH) test of 60 patients with ductal breast carcinoma stages I-IV (50). For the determination of DTH, the patients were injected with TF-antigen dissolved to 1% in PBS containing 0.25% phenol (the TF-Ag was prepared from neuraminidase treatment of MN on the surface of red blood cells probably expressed on glycophorin A) while controls received solvent alone or MN antigen dissolved to 1% in the solvent (50). 92% of ductal carcinoma patients with stage II to IV cancer and 79% with stage I cancer gave positive results; individuals without cancer gave negative results (50). Cellular immunity to TF-Ag was determined *in vitro* among majority of the skin tested population (not all) by leukocyte migration inhibition assay (LMI) in agarose plates (50) in which the white blood cells were isolated and reacted with or without TF-Ag (50). Results showed that patients who were negative for LMI were also negative for DTH (50). The discovery of TF-specific antigen in a reactive form in carcinoma but not in healthy tissues established this chemically well-defined antigen as carcinoma-associated (50).

6.2. Anti-TF-Ag antibodies and the relation to tumor prognosis.

Naturally occurring and induced antibodies have provided protection from viral, bacterial, and parasitic infections (50). Antibodies against tumor-associated antigens like TF-Ag are present within the body and have been shown to have some anti-cancer functions. Low levels of naturally occurring anti-TF-Ag antibodies have been correlated with tumor progression and aggressiveness (63). A study done by Somordin, *et al.* has shown that in gastric and breast cancer patients, the changes in anti-TF-Ag IgG are related to the disease progression (131) and in general, advanced gastric cancer is associated with lower anti-TF-Ag IgG (131). The median serum level of anti-TF-Ag IgG in patients with gastric cancer at stage III and IV (pools) was significantly lower than at stages I and II (131). Also lower levels of anti-TF-Ag IgG were observed in gastric cancer in cases with lymph node metastasis in comparison with those without lymph node metastasis (131). These findings implicate TF-Ag in tumor spread (131) due to the fact that as the TF-Ag bearing tumor increases in metastatic ability and aggressiveness, there are lower levels of anti-TF-Ag antibodies for them, enabling the cancer to thrive.

The levels of anti-TF-Ag antibodies serve as a means of determining the progression of the disease. The expression of TF-Ag on cancer cell surfaces is related to biological behavior and the prognosis of cancer patients (130). Additionally, low levels of TF-Ag-specific antibodies are associated with an increased risk for cancer (34, 39). These authors feel that low levels of anti-TF-Ag present a potential threat because in normal individuals, these anti-TF-Ag antibodies are believed to have an anti-cancer role to play. Decreased amounts of anti-TF-Ag thereby leaves one open for the development of cancer (130,34,50).

O. Kurtenkov and colleagues studied the humoral immune response of humans with breast cancer to carbohydrate antigens including TF-Ag before and after neoadjuvant chemotherapy regimens as well as in controls (healthy donors and patients with fibroadenoma). Higher levels of TF-Ag specific IgG antibody found before surgery were associated with a better survival time of stage II breast cancer patients (133). In another study done by this group, unlike the IgG levels, the anti-TF-Ag IgM antibody level showed no relation to the survival (129). The association of higher level of anti-TF-Ag IgG antibody with a better survival suggests that naturally occurring or adaptive immune responses to this epitope are of clinical importance and may be beneficial for the host (129). This shows the significant role of anti-TF-Ag IgG antibodies in the immunosurveillance of cancer (128). Natural anti-carbohydrate antibodies are normally of the IgM class, however the high level of anti-TF-Ag IgG observed in some patients may be a sign of acquired immune response which is indicative of the switching of the antibody to the IgG class (39).

It has also been found that infection with a particular microbe that causes increased anti-TF-Ag IgG may lead to a survival advantage when combined with certain cancers. One particular type of microbe which provides this survival advantage is the *Helicobacter pylori* (127) in combination with gastric cancer. Studies conducted by O. Kurtenkov and colleagues, found that in gastric cancer patients at stage I, significantly better survival for seropositive *H. pylori* patients was observed compared to *H. pylori* seronegative patients (127). Additionally, patients with higher levels of TF-Ag specific IgG antibody (strong responders) showed significantly and dramatically improved survival rate when compared to weak responders (127). The reason for this enhanced survival through the infection with this particular microbe is because the humoral immune response to *H. pylori* infection is similar to that in response to the TF-Ag (127). Hence, anti-TF-Ag antibodies are produced. Therefore, individuals already exposed to cancer and infected with this microbe have increased levels of the necessary anti-TF-Ag antibodies. The elevated levels of these antibodies seem to provide a survival advantage for these cancer patients.

Desai and colleagues did a study which involved using anti-TF-Ag antibodies as a means of carcinoma diagnosis. In this study, they developed a solid-phase immunofluorescence assay (*SPIA*-T) to determine anti-TF-Ag IgM and total IgM in human sera using solid phase polyacrylamide beads to which highly purified TF-Ag and anti-human IgM antibodies were covalently coupled, with fluorescent labeled IgM as the secondary antibody (50). The anti-TF-Ag IgM of $\leq$3mg/dl or $\geq$13mg/dl was found to be a positive indicator for cancer (50). With respect to the predictive value of anti-TF-Ag tests, it is noteworthy that positive anti-TF-Ag tests predicted carcinoma months to years before biopsy or x-rays (50) with 77% accuracy for clinical carcinoma within the 12 years of patients studied, where their biopsy and x-ray remained negative (50). The reason IgM was used is that the majority of human anti-TF-Ag antibodies are of the IgM subclass (50). The authors of this study feel that IgM is particularly useful for the early diagnosis of carcinomas, even before the development of diagnostic levels of markers commonly used for cancer diagnosis. Additionally, upon TF-Ag (purified from type O red blood cells) vaccination of patients with stages II to IV breast cancer, it was shown that 100% of stage IV and 94.4% of stage III survived 5 more years from the date of their primary tumor, and 87% of stage II breast cancer patients also survived 5 more years from the date of their primary tumor (50). This suggests that active stimulation of the immune system to produce anti-TF-Ag antibodies increases survival time of patients with cancer and may serve as a novel approach in cancer immunotherapy.

In conclusion, anti-TF-Ag antibodies are found naturally in the blood serum of normal individuals. It has been established that this antibody develops during weaning upon exposure to the natural intestinal flora which contains TF-Ag-positive bacteria (34). These antibody levels, however, are depressed upon the development of carcinoma. After surgery or chemotherapy the levels of antibody increase in patients with a good prognosis and do not increase in patients with a poor prognosis. This indicates that an effective TF-Ag vaccine would improve prognosis.

7. ROLE IN METASTASIS

Clinical correlations of TF-Ag with prognosis and *in vivo*, and *in vitro* studies have provided evidence that TF-Ag is related to metastasis. Mechanistic information concerning how TF-Ag could be involved in the adhesive steps of metastasis has been elucidated through the work of many individuals. The range of different studies, individually and collectively provide strong evidence for the role of TF-Ag in metastasis.

Clinically, the amount of TF-Ag on tumor cells correlates with prognosis in a number of cancer types, with increased surface TF-Ag being related to poorer prognosis in urinary, ovarian, lung, gastric, esophageal, breast and colon cancers. This implies that TF-Ag is involved in cancer spread although other factors could be involved (4-10, 55,133-138). While a 2006 study of tumor cells that had disseminated to the bone marrow in breast cancer patients showed no relation of metastasis to TF-Ag positivity or negativity, they found that TF-Ag negativity was associated with shortened disease free survival (136). Conversely,

Moriyama found that TF-Ag was associated with histologic grade and bone metastasis but not with survival (137). This clinical data does support a role for TF-Ag in metastasis and survival that may be complicated by other factors such as sensitivity to anti-estrogen therapy and other chemotherapies.

In 1998 Lehr and Pienta (138) showed that adhesion of a prostate cancer cell line to human bone marrow endothelial cells was mediated by a carbohydrate – galectin 3 interaction. Also in 1998, Shigeoka et al. (139) performed *in vivo* analysis in a mouse model, and showed that neuraminidase treatment of Colo26 cells increased the amount of TF-Ag on the tumor cell surface and increased the frequency of liver metastasis formed by these cells three-fold. Antibody to TF-Ag inhibited the development of liver metastasis while control antibody did not. This indicated that the liver localization was due to the TF-Ag expression. An associated clinical study showed that tumors with increased TF-Ag expression were more likely to metastasize to the liver (55). The authors hypothesized that tumor cell TF-Ag bound to the sialoglycoprotein receptor on hepatocytes and Kuppfer cells in the liver, and that this interaction was involved in metastasis.

In studies with our IgG_3 monoclonal antibody, JAA-F11, we observed that lung metastasis were reduced by over 50% in a 4T1 mouse metastatic breast cancer model, presumably by blocking TF-Ag mediated adhesive properties required for lung metastasis (140). The antibody was transferred after the primary tumor was established, so this activity was not due to an effect on primary tumor development. Other studies have shown that JAA-F11 is not cytolytic by antibody dependent cell mediated cytotoxicity, nor by complement directed cytotoxicity, so this effect was due to blocking steps of metastasis, not due to cytolytic effect. In unpublished studies from our laboratory, a derivative cell line of 4T1 mouse metastatic breast cancer, which had increased 2-3 sialyltransferase activity and decreased expression of TF-Ag on its surface, did not metastasize. This indicates that a sialic acid linked to the Gal blocks interaction of the TF-Ag with ligands involved in metastasis.

In 1997, Quinn's laboratory, using a phage display library discovered a peptide (P30) which bound to TF-Ag (141). In further studies by Glinsky et al., this peptide blocked homotypic aggregation of MDA-MB-435 cells in a dose dependent manner indicating that tumor cells stuck to each other in a TF-Ag dependent manner (142). P30 also inhibited binding of tumor cells to the endothelium (a process involved in metastasis), indicating TF-Ag was involved in this also. The peptide also blocked TF-Ag-galectin-3 mediated stable and rolling adhesion in microvessels (143). During these experiments, clustering of galectin-3 on the endothelial cells at the interface with the tumor cells was observed and this indicated a role for galectin-3 in this adhesion. In a collaborative study, Deutscher confirmed the role of galectin-3 in tumor metastasis using a synthetic peptide which bound to the carbohydrate recognition domain of galectin-3, but not other galectins or plant lectins. This peptide also inhibited the interaction of galectin-3 and TF-Ag, and inhibited heterotypic adhesion of tumor cells to the endothelium and homotypic adhesions of tumor cells to each other (144).

In related studies in 2001, Glinskii (145) showed that the interaction of TF-Ag containing glycoproteins with endothelial cells causes mobilization of galectin-3 to the cell surface. Synthetic compounds which either masked or mimicked TF-Ag could block the interaction of tumor cells with the endothelial cells. These studies involved measurements of the amount of tumor cell adhesion to human umbilical vein endothelial cells (HUVECs), and human bone marrow endothelial cells (HBMECs). Monoclonal antibody to TF-Ag (JAA-F11) inhibited reactivity of tumor cells with either of these human endothelial cell types. The tumor cells utilized in this study were DU-145 human prostate cells and MDA-MB 435 cells, (most likely breast cancer cells although with some controversy concerning whether they could be human melanoma cells) (193,194). In this work, Glinsky proposes that TF-Ag and galectin-3 interaction with galectin-3 mobilization to the endothelial cell surface is involved in the first step in a two- step tumor cell- endothelial cell interaction. He suggests that the second step is an integrin mediated locking. Evidence for the role of TF-Ag in the initial interaction of a two-step process is that under flow conditions, which simulate blood flowing through the vasculature, anti-TF-Ag antibody (JAA-F11) can block the adhesion of tumor cells to the endothelium, whereas anti-integrin antibodies (alpha-4 and beta-2) do not block this interaction at all. Anti-integrin antibodies, can, however, inhibit adhesion of PC-3 prostate cancer cells to tumor cells incubated in place for 1 hour with endothelial cells (145).

In 2003, studies with MDA-MB-468 tumor cells, a TF-Ag low and galectin-3 low analog of MDA-MB-435 did not cause upregulation and migration of endothelial cell galactin-3 levels. After MDA-MB-435 tumor cells bind to the endothelial cells, there was an up-regulation of surface galactin-3 on the side opposite the endothelial cells. This up-regulation of galectin-3 on the tumor cell on the side away from the endothelium, was associated with tumor cell homotypic aggregation at the endothelial site (146). This would indicate that homotypic aggregation of tumor cells at the endothelial cell surface is a third step in the multi-step process of TF-Ag's involvement in the metastatic process.

Additional work in 2004 by Glinskii showed that endothelial cell interactions with TF-Ag on the tumor cell surface brought about the up-regulation of endothelial cell surface galectin-3 which affected the tumor cell velocity and sticking to the endothelium. TF-Ag positive tumor cell velocity in the perfused porcine dura mater model reduced to approximately one tenth of the previous velocity, and the percent of tumor cells rolling along the vascular surface rather than flowing through the arterioles increased from 3.3% to 23.4%. TF-Ag negative cells did not decrease in velocity nor did their adhesion increase. The surface up-regulation of galectin-3 could be caused by asialofetuin as a TF-Ag source, and the upregulation of surface galectin-3 and the subsequent velocity changes could be blocked by JAA-F11 antibody to TF-Ag (146). In 2005, Glinskii's

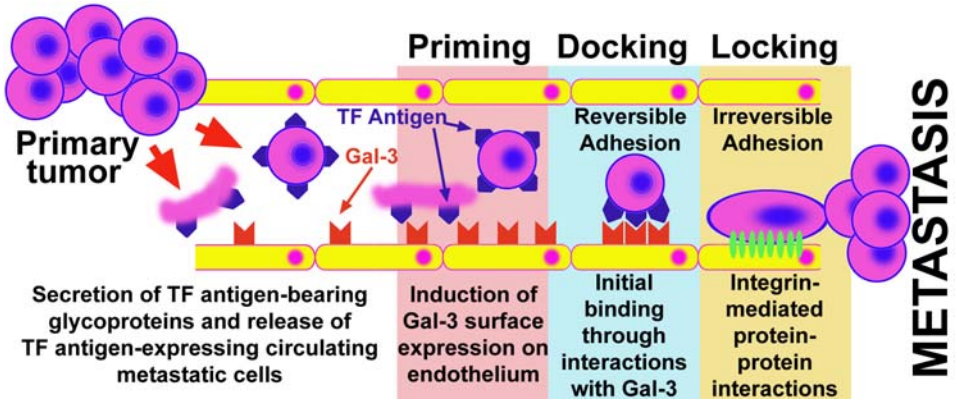

Figure 2. Schematic representation of TF antigen participation in adhesion of metastatic cancer cells to the endothelium. Circulating TF antigen-bearing cancer-associated glycoproteins and metastatic tumor cells induce the mobilization of the galectin-3 to the surface of endothelial cells (priming stage). TF antigen-expressing malignant cells bind to the endothelium through TF antigen/galectin-3 interactions (docking stage) allowing sufficient time for stabilizing integrin-mediated adhesion events to take place (locking stage) Reproduced with permission from reference 133.

experiments (147) showed that the adhesive interactions are necessary for tumor cell arrest on endothelium of the bone and the lungs for both DU-145 human prostate cancer and MDA-MB-435 human melanoma cells. Arrest of tumor cells could be blocked by JAA-F11 antibody to TF-Ag, antibody to Galectin-3, modified citrus pectin, and lactulosyl-L-Leucine. A summary figure by Glinski showing the role of TF-Ag in metastasis adapted from reference 133 is shown in Figure 2.

In 2007, (84, 148) Yu showed that circulating galectin-3 increases the binding of circulating MUC-1 positive breast and colon tumor cells to the endothelial cell surface, but does not affect binding of MUC-1 negative cells. MUC-1 is a tumor associated mucin that carries many TF-Ag epitopes. Adhesion of the tumor cells was reduced through treatment with O-glyconase which is specific for removing unsubstituted TF-Ag from serine or threonine residues. A transfected cell line which contained MUC-1 with sialylated TF-Ag rather than unsubstituted TF-Ag did not stick to the endothelial cell surface unless the sialic acid was removed with sialidase. These results strongly indicate a role for galectin-3 and TF-Ag in cancer cell adhesion to the vascular endothelium. Antibodies to E-Selectin and CD44H blocked this binding. CD44 also carries TF-Ag epitopes (29,113, 149). CD44 has been found to be involved in skeletal metastasis, and another carrier of the TF-Ag, CD164 has been implicated in prostate cancer cell adhesion to the bone marrow endothelium (149, 150-154). Contrary to the model suggested by Glinskii with TF-Ag on MUC-1 and other carrier molecules directly interacting with galectin-3 on the endothelium, Yu (84) saw movement of the MUC-1 away from the tumor cell-endothelial cell contact. Yu suggests that this redistribution removes a MUC-1 shield from the adhesion molecules that subsequently interact. These differences have not been resolved conclusively. In 2010, Zhao in Yu's group showed that the MUC-1 TF-Ag mediated galectin-3 interaction enhanced homotypic tumor cell aggregation (148). This study also showed that this TF-Ag mediated homotypic aggregation decreased tumor cell anoikis. Anoikis is cell death due to apoptosis caused by loss of anchorage.

In 2010 Gassman, (85) utilized an *in situ* model in a ventilated and perfused lung to study tumor cell metastasis to the lung. An anesthetized rat was mechanically ventilated and the anterior chest wall was removed so that the lung vasculature could be observed under a fluorescence microscope. Tumor cells fluorescently labeled with Calcein AM could be observed sticking to the vasculature in this model. Their work showed that tumor cell adhesion to the pulmonary microvasculature could be inhibited by pre-treatment with JAA-F11 antibody to TF-Ag, or by neuraminidase type V treatment of the tumor cells. Prior perfusion of the rats with fucoidan to block L- and P-selectin also inhibited binding, but the specificity of fucoidan interaction is too broad to make a conclusion about a specific P- or L-selectin effect. Neither antibodies to integrins nor antibodies to galectin-3 blocked this binding. Antibodies were pre-incubated with the tumor cells and the cells were washed prior to perfusion in the lungs, so the lung vascular endothelial cells were not exposed to the antibody.

In summary, there is much evidence that shows that TF-Ag plays a role in tumor cell metastasis. Clinical indications of the importance of TF-Ag in metastasis have come through evidence of poorer prognosis in urinary, ovarian, lung, gastric, esophageal, breast and colon cancers. Experimental evidence using *in vivo and in vitro* models has also supported the key role that TF-Ag plays metastasis in utilizing TF-Ag positive melanoma cells and prostate, breast, colon cancer cells. Since TF-Ag plays a mechanistic role in the cancer spread an immune response could have blocking function, inhibiting metastasis, as well as cytolytic function. In addition, a mechanistic role for the antigen decreases the chance that antigen negative cancer cells will develop. The current information concerning the role of TF-Ag in the development of metastasis is a strong indication that if antibody to TF-Ag was developed in the patient it would improve prognosis.

8. VACCINE

TF-Ag is an ideal vaccine candidate to prevent or treat cancer because the TF-Ag is expressed in various carcinomas, and can be applied to a large population of cancer patients (45,155,156). In addition, since TF-Ag plays a role in metastasis, downregulation of this antigen, if it did occur due to antibody therapy, would result in the remaining cancer cells having a less metastatic phenotype. During the last 20 years, the use of TF-Ag as a cancer vaccine has been investigated by various groups (45,19,42,157-159). Among the first were Springer and coworkers who conducted a long term intradermal vaccination of 32 patients with advanced breast carcinomas, using vaccine consisting of human group O red blood cell membranes containing HLA-free TF/Tn Ag most likely attached to glycophorin A with the adjuvant $Ca_3(PO_4)_2$ plus a trace of phosphoglycolipid A hyperantigen, i.e., *S. typhi* vaccine (USP) (45). All 32 patients survived for at least 5 years after the vaccinations and immune responses in the patients were measured in delayed-type hypersensitivity reactions. Since then the number of those engaged in developing TF-Ag cancer vaccines has increased and several groups have synthetically or chemically modified TF-Ag in order to improve the immune response.

Fung *et al* and MacLean *et al* both used a synthetic immunogen of TF-Ag conjugated to keyhole limpet haemocyanin (KLH (158, 159). Fung et al used a synthetic tumor-associated glycoconjugate (S-TAG) vaccine that is composed of TF-Ag coupled to KLH, cyclophosphamide emulsified in Ribi adjuvant containing trehalose dimycolate and monophosphoryl lipid A. Long-term survival of mammary adenocarcinoma (TA3-Ha) bearing-mice in both preventive and therapeutic settings was seen as compared to control mice receiving the vaccine without Ribi adjuvant or cyclophosphamide (158). The cyclophosphamide was added to down-regulate T suppressor cell activity. Surviving mice that received a subsequent lethal, high-dose tumor challenge produced high anti-TF-Ag (alpha) IgG antibody titers (1:1280) and strong delayed-type hypersensitivity (DTH) responses to the TF-Ag-alpha epitope (158). In a phase I study, Maclean *et al* showed that ten ovarian cancer patients who received cyclophosphamide before being immunized with a synthetic TF-Ag antigen conjugated to KLH plus DETOX adjuvant developed moderate to strong DTH reactions at the vaccination sites. Nine of the ten patients showed an increase in anti-TF-Ag alpha IgM antibodies above pre-existing IgM antibodies. IgG anti-TF-Ag alpha were also generated in the same nine patients while eight of them produced IgA anti-TF-Ag alpha antibodies. Xu et al showed that synthetic glycopeptides vaccines containing TF-Ag linked to a major T cell receptor (TcR) contact residue and having high affinity for MHC class 1 molecules are immunogenic *in vivo* and can induce anti-TF-Ag cytotoxic T cells in mice (160). The mammary tumor cell lines TA3/Ha that expressed TF-Ag was used in a ^{51}Chromium release assay to determine anti-TF-Ag cytotoxic T cells activity (160). A vaccine consisting of synthetic TF-Ag conjugated with crotyl linker to KLH was used to immunize colon cancer patients. Although the vaccine induced a high IgM and IgG antibody responses against the synthetic TF-Ag, the IgM antibodies showed weak reactivity, whereas, the IgG antibodies were almost unreactive when tested against the natural TF-Ag (161). This may have been related to the use of the very short linker region to link the disaccharide to the protein carrier.

In order to improve immune response, TF-Ag has also been modified in many ways. Some groups have constructed combinations of glycan antigens that include TF-Ag residues linked to unique carriers. Slovin and coworkers reported that a vaccine consisting of a synthetic TF-Ag in a clustered formation coupled to KLH and administered with the saponin adjuvant QS21 to twenty prostate cancer patients in a phase I clinical trial was able to induce high-titer IgM and IgG antibodies against TF-Ag in all patients. In his studies, 33% of the patients showed a favorable clinical response as measured by a decrease in prostate specific antigen (PSA) log slopes. (162). Saponin QS21, a glycoside that is derived from the bark of *Quillaja saponaria* Molina, is able to stimulate both Th1 immune response and the production of CTLs and has been used as adjuvants in vaccine formulations against infections and cancers (163, reviewed in 164). QS21 was found to be the most potent in inducing antibody response against conjugate vaccines (165, 166). The vaccine was safe and the patients did not show any clinical signs of autoimmunity (162).

To determine whether immune response could be further enhanced with stimulation by multiple antigens, a hexavalent vaccine that included GM2, Globo H, Lewis^y, glycosylated MUC-1-32mer and Tn and TF in a clustered formation, conjugated to KLH and mixed with QS-21 was administered in a Phase II setting to 30 high-risk patients (167). Antibody titers against at least two of the six antigens were elevated in all 30 patients. Compared to individual monovalent trials, the antibody titers against several of the antigens were actually lower. In a pilot study, Sabbatini et al used a heptavalent vaccine that included a cluster of three TF-Ag conjugated to KLH and mixed with adjuvant QS21 to immunize eleven patients with epithelial ovarian, fallopian tube, or peritoneal cancer (168). Nine of the vaccinated patients had antibody responses against at least one of these antigens, while eight of the nine patients had antibodies against three of the seven antigens. Tn-MUC-1 and TF-Ag were the most potent immunogens in the heptavalent vaccine (168).

Roder *et al* immunized mice with a vaccine consisting of synthetic epithelial mucin MUC-1 with the TF-Ag or a difluoro analogue coupled to tetanus toxoid (TTox). This vaccine induced antibodies that were selectively directed against the tumor-associated MUC-1 structures and which strongly bound to MCF-7 breast cancer cells (169). A bioengineered glycoconjugate containing the TF-Ag disaccharide mixed with Freund's adjuvant was shown to induce an immune response and this immune response inhibited tumor growth in an *in vivo* colon carcinoma CT26 mouse model (170). Tumors from mice immunized with the bioengineered glycoconjugates

were 36% and 39% smaller compared to tumors from mice immunized with KLH alone.

Another approach to improve the immunogenicity of TF-Ag containing vaccine is using self-adjuvanting multicomponent vaccines. These self-adjuvanting vaccines, made up of a tumor-associated carbohydrate antigen, a helper T-cell epitope, and an immune-stimulating agent such as a TLR agonist have been shown to induce immune responses in mice (171, 172). Boons and co-workers showed that multicomponent vaccines containing a MUC-1 peptide bearing a single Tn antigen, the Toll-like receptor 2 (TLR2) ligand, $Pam_3CysSerK_4$ and a helper T-cell epitope derived from the polio virus induced high titers of IgG antibodies in mice which were able to recognize MUC-1 antigen on MCF7 cancer cells (171). Recently, Wilkinson and co-workers demonstrated that a synthetic multicomponent vaccine comprised of a tetanus toxin-derived helper T cell epitope incorporated between a glycosylated MUC-1 bearing multiple copies of TF-Ag and the TLR2 agonist $Pam_3CysSer$ induced high IgG antibodies in mice (172).

The immunogenicity of carbohydrate antigens can also be raised using peptide mimicry of the carbohydrate epitope (173, 174, 175). A number of immunization studies using peptide mimics of carbohydrate antigens have shown that these peptide mimics were able to induce an enhanced T-cell dependent (TD) immune response (176-179). Encouraged by the results of these studies, our lab has developed peptide mimics of the TF-Ag. We began by biopanning a 12-mer phage display library to isolate phage that reacted with our monoclonal antibody to TF-Ag, JAA-F11 that had been developed earlier (180, 79). We used these peptide mimics together with alum and *Bordetella pertussis* to immunize mice and IgM and IgG TF-Ag antibodies were produced (180). We have begun structural studies of two of the peptide mimics using X-ray crystallography to identify common interactions with carbohydrate–antibody complex to lead to improved peptide mimics to create the best possible peptide mimic for TF-Ag to be used as vaccine in treating cancer patients. Our collaborators, including A. Gulick, R. Woods, S. Jadey, M. Tessier, and J. Heimburg-Molinaro are utilizing binding data, X-ray crystal structure of JAA-F11 Ab and computational docking to better understanding of the interactions between the JAA-F11 TF-Ag alpha restricted antibody and TF-Ag. This will facilitate vaccine development, especially if a TF-Ag alpha response is required. This information, combined with NMR studies of the peptide mimic binding to the JAA-F11 should allow for the production of the optimal peptide mimic for further vaccine development.

In the future, we will attempt to use constructs containing the TF-Ag mimicking peptides improved as described above linked to one or more immune-stimulating agents including C3d, Toll-like receptor 9 (TLR9) agonists such as cytosine-phosphate-guanosine oligodeoxy-nucleotides (CPG ODNs), and cytokines such as IL-12. Adjuvants are a key component of effective vaccines and we will investigate the ability of these adjuvants to augment immune responses induced by the TF-Ag peptide mimics. Synthetic TLR9 agonists have strong adjuvant activities that include stimulation of antigen-specific B cells, enhanced IgG class switch, and cytotoxic T lymphocytes generation (181, 182). Many clinical trials are currently being carried out to evaluate TLR9 agonists as adjuvants against infections and in cancer treatment (183-186). IL-12 which promotes the development of Th1 cells and induces the production of Th1-type antibody production has been shown to be a highly effective vaccine adjuvant (187, 188). C3d can facilitate activation of B cells without T-cell help by targeting CD21, the C3d receptor, on splenic marginal zone B cells, and follicular dendritic cells (189). C3d has been used as molecular adjuvant for vaccines encoding a variety of pathogen-derived antigens (190) and also for tumor immunotherapy (191). In addition, nanoparticle constructs of TF-Ag have been developed by Barchi, and we will continue collaboration with this group to determine if nanoparticle presentation of TF-Ag will improve immunogenicity (192). These additional vaccine strategies could improve the immunogenicity of the peptide mimics toward the TF-Ag tumor epitope.

In summary, the Thomsen-Friedenreich glycoantigen (TF-Ag) is on the surface of breast, colon, bladder, prostate and other carcinomas and is involved in adhesion and metastasis. Data strongly indicate that this will be an important target for passive and active immunotherapy. A particularly important patient population is the triple negative (estrogen and progesterone receptor negative and Her2 negative) breast cancer patient. This patient group is important because targeted hormonal therapy and targeted anti-growth factor receptor therapies do not decrease tumor burden in these patients. These patients, in general, are younger and have poorer prognosis than breast cancer patients whose cancer cells have these markers. Metastasis inhibition experiments show that anti-TF-Ag immune therapy may have a role in breast, colon and prostate cancer as well as in melanoma. In addition, immunohistochemistry studies have found TF-Ag on the surface of cancers of the bladder, breast, colon, lung, liver, ovary, prostate and stomach, suggesting that TF-Ag may be an immunotherapeutic target for a wide range of cancers.

We believe that the highly specific JAA-F11 antibody to this antigen can be used to detect whether the tumor has spread and can be used as adjunct therapy to improve survival of patients with these TF-Ag expressing tumors. In previous experiments, JAA-F11 has been shown to decrease metastasis to the lung and increase survival in a mouse breast cancer *in vivo* model. Iodine-124 labeled JAA-F11 Ab in *in vivo* micro positron emission tomography (microPET) showed tumor localization in the mouse breast tumor model. Preliminary results with JAA-F11 in a triple negative human breast cancer cell line shows positive results *in vitro,* using a whole cell enzyme immunoassay and *in vivo*, using microPET imaging. Collectively, these results indicate that JAA-F11 shows promise as a method to find tumor metastasis and that it has potential to be used with chemotherapy to block metastasis to the lung and decrease tumor burden. These results

strongly support Phase I trials using radiolabeled JAA-F11 to determine whether that antibody successfully localizes to human tumors. This information will be used to develop the antibody as a product for use in detecting whether or not the tumor has spread. Subsequent experiments would involve humanization of the antibody and utilization of the humanized JAA-F11 antibody as a product for adjunct therapy along with chemotherapy for the cancer patient

Vaccine development, targeting TF-Ag, for active immunotherapy is also strongly supported in order to develop a response in the cancer patient that will prevent spread of the tumor and kill the tumor cells. Complex molecular modeling experiments combined with novel strategies to improve immunogenicity should allow these experiments to successfully create this vaccine.

9. ACKNOWLEDGEMENT

Thank you to James R. Olson for proofreading and encouragement throughout all the experiments described. Thank you to Susan Morey for excellent technical support and mentoring support of the graduate students. Thank you to all my collaborators, especially Jamie Heimburg-Molinaro, Joseph Barchi, Rob Woods, and Rene Roy. Recognition and thanks for the grant support of CDMRP W81XWH-04-1-0342, Oncologic Foundation of Buffalo, UB STOR Product Development Fund, NIAID R15 AI 49210-01 Oishei Foundation, the King Saud University Chairs Research Fund and the valuable support of the Consortium of Functional Glycomics National Institute of General Medical Sciences grant GM62116.

10. REFERENCES

1. O.Z. Thomsen: Ein vermehrungsfahiges Agens als verandererdes isoagglutinatorischen verhaltens der roten Blutkorperchen,eine bisher unbekannta Quelle der Fehlbestimmungen. *Zeitschrift fuÈr ImmunitaÈts Forschung,* 52, 85-90 (1927).

2.V.Friedenreich:The Thomsen hemagglutination phenomenon. Levin and Munksgaard, Copenhagen, Denmark. 1930.

3. E. Neter: Bacterial Hemagglutination and Hemolysis. *Microbiol. Mol. Biol. Rev.* 20, 166-188 (1956)

4. Lu-Gang Yu: The oncofetal Thomsen-Friedenreich carbohydrate antigen in cancer progression. *Glycoconj J* 24, 411-420 (2007)

5. S. E. Baldus, T. K. Zirbes, J. Glossmann, S. Fromm, F. G. Hanisch, S. P. Monig, W. Schroder, P. M. Schneider, U. Flucke, U. Karsten, J. Thiele, A. H. Holscher, and H. P. Dienes: Immunoreactivity of monoclonal antibody BW835 represents a marker of progression and prognosis in early gastric cancer. *Oncology* 61, 147-155 (2001)

6. Y. S. Chung, Y. Yamashita, Y. Kato, B. Nakata, T. Sawada, and M. Sowa: Prognostic significance of T antigen expression in patients with gastric carcinoma. *Cancer* 77, 1768-1773 (1996)

7. E. Dabelsteen: Cell surface carbohydrates as prognostic markers in human carcinomas. *J Pathol* 179, 358-369 (1996)

8. H. Fukatsu, H. Nonomura, Y. Miyagawa, M. Waki, Y. Hatano, S. Hiraiwa, T. Muramatsu, E. Nishikawa, Y. Yamada, and T. Satoh: The study on cell surface antigens in epithelial tumor of the upper urinary tract: ABH-isoantigen and Thomsen-Friedenreich antigen. *Hinyokika Kiyo* 35, 949-954 (1989)

9. M. Ghazizadeh, T. Oguro, Y. Sasaki, K. Aihara, T. Araki, and G. F. Springer: Immunohistochemical and ultrastructural localization of T antigen in ovarian tumors. *Am J Clin Pathol* 93, 315-321 (1990)

10. B. R. Juhl: Blood group antigens in transitional cell tumours of the urinary bladder. An immunohistochemical study. *Dan Med Bull* 41, 1-11 (1994)

11. G. F. Springer, Y. Nagai, and H. Tegtmeyer: Isolation and properties of human blood-group NN and meconium-Vg antigens. *Biochemistry* 5, 3254-3272 (1966)

12.G. F. Springer, P. R. Desai, and I. Banatwala: Blood group MN specific substances and precursors in normal and malignant human breast tissues. *Naturwissenschaften* 61, 457-458 (1974)

13. G. F. Springer, P. R. Desai, and E. F. Scanlon: Blood group MN precursors as human breast carcinoma-associated antigens and "naturally" occurring human cytotoxins against them. *Cancer* 37, 169-176 (1976)

14. G. F. Springer, P. R. Desai, M. S. Murthy, and E. F. Scanlon: Delayed-type skin hypersensitivity reaction (DTH) to Thomsen-Friedenreich (T) antigen as diagnostic test for human breast adenocarcinoma. *Klin Wochenschr* 57, 961-963 (1979)

15. G. F. Springer, P. R. Desai, M. S. Murthy, and E. F. Scanlon: Human carcinoma-associated precursor antigens of the NM blood group system. *J Surg Oncol* 11, 95-106 (1979)

16. G. F. Springer, P. R. Desai, M. S. Murthy, H. Tegtmeyer, and E. F. Scanlon: Human carcinoma-associated precursor antigens of the blood group MN system and the host's immune responses to them. *Prog Allergy* 26, 42-96 (1979)

17. G. F. Springer, P. R. Desai, M. S. Murthy, H. J. Yang, and E. F. Scanlon: Precursors of the blood group MN antigens as human carcinoma-associated antigens. *Transfusion* 19, 233-249 (1979)

18. P. R. Desai and G. F. Springer: Sialylation of Thomsen-Friedenreich (T) and blood type NM antigens by

transferases in human sera measured by 14C NAN uptake. *J Immunogenet* 7, 149-155 (1980)

19..G. F. Springer, M. S. Murthy, P. R. Desai, and E. F. Scanlon: Breast cancer patient's cell-mediated immune response to Thomsen-Friedenreich (T) antigen. *Cancer* 45, 2949-2954 (1980)

20. G. F. Springerand H. Tegtmeyer: On the origin of anti-Thomsen-Friedenreich (T) antibodies. *Naturwissenschaften* 67, 317-318 (1980)

21. G. Uhlenbruck: The Thomsen-Friedenreich (TF) receptor: an old history with new mystery. *Immunol Commun* 10, 251-264 (1981)

22. G. F. Springer and H. Tegtmeyer: Origin of anti-Thomsen-Friedenreich (T) and Tn agglutinins in man and in White Leghorn chicks. *Br J Haematol* 47, 453-460 (1981)

23. G. F. Springer and P. R. Desai: Extent of desialation of blood group MM, NN, and MN antigens required for reactivity with human anti-T antibody and Arachis hypogaea lectin. *J Biol Chem* 257, 2744-2746 (1982)

24. G. F. Springer and P. R. Desai: Detection of lung- and breast carcinoma by quantitating serum anti-T IgM levels with a sensitive, solid-phase immunoassay. *Naturwissenschaften* 69, 346-348 (1982)

25. G. F. Springer and P. R. Desai: Similarity between Ca antigen and Thomsen-Friedenreich antigen. *Lancet* 2, 660 (1982)

26. G. F. Springer, S. M. Murthy, P. R. Desai, W. A. Fry, H. Tegtmeyer, and E. F. Scanlon: Patients' immune response to breast and lung carcinoma-associated Thomsen-Friedenreich (T) specificity. *Klin Wochenschr* 60, 121-131 (1982)

27.W. A. Fry, G. F. Springer, and P. R. Desai: Lung cancer patients' autoimmune responses to Thomsen-Friedenreich (T) antigen: diagnostic utility. *Klin Wochenschr* 61, 817-818 (1983)

28. R. L. Goodale, G. F. Springer, J. G. Shearen, P. R. Desai, and H. Tegtmeyer: Delayed-type cutaneous hypersensitivity to Thomsen-Friedenreich (T) antigen in patients with pancreatic cancer. *J Surg Res* 35, 293-297 (1983)

29. J. M. Ignatoff, G. F. Springer, M. D. Blum, H. Tegtmeyer, and P. R. Desai: Patients mount an autoimmune response to their bladder cancer-associated t antigen. *Naturwissenschaften* 70, 42 (1983)

30. V. Schirrmacher, R. Dzarlieva, P. Altevogt, M. Fogel, C. A. Waller, J. W. Dennis, G. F. Springer, I. Vlodavsky, M. Kramer, and R. Cheingsong-Popov: Phenotypic and genotypic differences between high- and low-metastatic related tumor lines and the problem of tumor progression and variant generation. *Symp Fundam Cancer Res* 36, 81-99 (1983)

31. G. F. Springer: Blood group T and Tn antigens are universal, clonal, epithelial cell-adhesive, autoimmunogenic carcinoma markers. Prog Clin Biol Res 133, 157-166 (1983).

32. G. F. Springer: Tn and T blood group precursor antigens are universal, clonal, epithelial cell-adhesive, autoimmunogenic carcinoma (CA) markers. Naturwissenschaften 70, 369-370 (1983)

33.G. F. Springer, P. R. Desai, W. A. Fry, R. L. Goodale, J. G. Shearen, and E. F. Scanlon: T antigen, a tumor marker against which breast, lung and pancreas carcinoma patients mount immune responses. Cancer Detect Prev 6, 111-118 (1983)

34. G. F. Springer: T and Tn, general carcinoma autoantigens. Science 224, 1198-1206 (1984)

35. G. F. Springer, W. A. Fry, P. R. Desai, R. A. Semerdjian, H. Tegtmeyer, C. G. Neybert, and E. F. Scanlon: Further studies on the detection of early lung and breast carcinoma by T antigen. Cancer Detect Prev 8, 95-100 (1985)

36. G. F. Springer, P. R. Desai, M. K. Robinson, H. Tegtmeyer, and E. F. Scanlon: The fundamental and diagnostic role of T and Tn antigens in breast carcinoma at the earliest histologic stage and throughout. Prog Clin Biol Res 204, 47-70 (1986)

37. H. Clausen, M. Stroud, J. Parker, G. Springer, and S. Hakomori: Monoclonal antibodies directed to the blood group A associated structure, galactosyl-A: specificity and relation to the Thomsen-Friedenreich antigen. Mol Immunol 25, 199-204 (1988)

38. D. M. Thomson, G. F. Springer, P. R. Desai, R. Scanzano, M. Gubersky, and G. Shenouda: Comparison by leukocyte adherence inhibition of human immune response to cancer-associated immunogens, Thomsen-Friedenreich (T) and Tn, myelin basic protein, and organ-specific cancer neoantigens. Clin Immunol Immunopathol 49, 231-241 (1988)

39. P. R. Desai, L. H. Ujjainwala, S. C. Carlstedt, and G. F. Springer: Anti-Thomsen-Friedenreich (T) antibody-based ELISA and its application to human breast carcinoma detection. J Immunol Methods 188, 175-185 (1995)

40. G. F. Springer, P. R. Desai, M. Ghazizadeh, and H. Tegtmeyer: T/Tn pancarcinoma autoantigens: fundamental, diagnostic, and prognostic aspects. Cancer Detect Prev 19, 173-182 (1995)

41..G. F. Springer: T and Tn pancarcinoma markers: autoantigenic adhesion molecules in pathogenesis,

prebiopsy carcinoma-detection, and long-term breast carcinoma immunotherapy. *Crit Rev Oncog* 6, 57-85 (1995)

42. G. F. Springer, P. R. Desai, B. D. Spencer, H. Tegtmeyer, S. C. Carlstedt, and E. F. Scanlon: T/Tn antigen vaccine is effective and safe in preventing recurrence of advanced breast carcinoma. *Cancer Detect Prev* 19, 374-380 (1995)

43.Y. Cao, P. Stosiek, G. F. Springer, and U. Karsten: Thomsen-Friedenreich-related carbohydrate antigens in normal adult human tissues: a systematic and comparative study. *Histochem Cell Biol* 106, 197-207 (1996)

44.D. Avichezer, G. F. Springer, B. Schechter, and R. Arnon: Immunoreactivities of polyclonal and monoclonal anti-T and anti-Tn antibodies with human carcinoma cells, grown in vitro and in a xenograft model. *Int J Cancer* 72, 119-127 (1997)

45. G. F. Springer: Immunoreactive T and Tn epitopes in cancer diagnosis, prognosis, and immunotherapy. *J Mol Med* 75, 594-602 (1997)

46. B. L. Wang, G. F. Springer, and S. C. Carlstedt: Quantitative computerized image analysis of Tn and T (Thomsen-Friedenreich) epitopes in prognostication of human breast carcinoma. *J Histochem Cytochem* 45, 1393-1400 (1997)

47. B. L. Wang, G. F. Springer, and L. C. Harwick: T (Thomsen-Friedenreich) and Tn epitope location and their spatial relations to adhesion plaques on human breast carcinoma cells: immunogold-silver staining studies at scanning electron microscopic level. *J Submicrosc Cytol Pathol* 30, 503-509 (1998)

48. T. Kishikawa, M. Ghazizadeh, Y. Sasaki, and G. F. Springer: Specific role of T and Tn tumor-associated antigens in adhesion between a human breast carcinoma cell line and a normal human breast epithelial cell line. *Jpn J Cancer Res* 90, 326-332 (1999)

49. George Garratty: A tribute to Georg Springer, MA, MD, DSc (Hon)--who laid a foundation for using the relationship of blood groups to disease as a basis for the diagnosis and treatment of patients. *Transfus Med Rev*. 2000 Oct;14(4):289-90 (2000)

50. P. R. Desai Immunoreactive T and Tn antigens in malignancy: role in carcinoma diagnosis, prognosis, and immunotherapy. *Transfus Med Rev*. (4):312-25 (2000)

51. E. A. Abdi, V. J. Kamitomo, T. A. McPherson, Z. Catz, G. Boniface, B. M. Longenecker, and A. A. Noujaim: Radioiodinated peanut lectin: clinical use as a tumour-imaging agent and potential use in assessing renal-tubular function. *Eur J Nucl Med* 11, 350-354 (1986)

52. S. E. Baldus, F. G. Hanisch, G. M. Kotlarek, T. K. Zirbes, J. Thiele, J. Isenberg, U. R. Karsten, P. L. Devine, and H. P. Dienes: Coexpression of MUC1 mucin peptide core and the Thomsen-Friedenreich antigen in colorectal neoplasms. *Cancer* 82, 1019-1027 (1998)

53. S. O. Demichelis, C.G. Alberdi, W. Servi, M. Isla-Larrain, A. Segal-Eiras, M.V. Croce: Comparative Immunohistochemical Study of MUC1 and Carbohydrate Antigens in Breast Benign Disease and Normal Mammary Gland. *Applied Immunohistochemistry & Molecular Morphology*: 18 , 41-50 (2010)

54. Sankaranarayanan Kannan, Reddi A Lakku, Devaraj Niranjali, Kamala Jayakumar, Arulraj H Steven, Taralakshmi VV, Chandramohan S, Ramathilakam Balakrishnan, Christian Schmidt and Devaraj Halagowder Expression of peanut agglutinin-binding mucin-type glycoprotein in human esophageal squamous cell carcinoma as a marker *Molecular Cancer* **2**:38-48 (2003)

55. Y. Cao, U. R. Karsten, W. Liebrich, W. Haensch, G. F. Springer, and P. M. Schlag: Expression of Thomsen-Friedenreich-related antigens in primary and metastatic colorectal carcinomas. A reevaluation. *Cancer* 76, 1700-1708 (1995)

56.Yi Cao, Anette Merling, Uwe Karsten, Steffen Goletz, Michael Punzel, Regine Kraft, Gunter Butschak, and Reinhard Schwartz-Albiez: Expression of CD175 (Tn), CD175s (sialosyl-Tn) and CD176 (Thomsen-Friedenreich antigen) on malignant human hematopoietic cells. *Int J Cancer* 123, 89-99 (2008)

57.S. D. Ryder, J. A. Smith, and J. M. Rhodes: Peanut lectin: a mitogen for normal human colonic epithelium and human HT29 colorectal cancer cells. *J Natl Cancer Inst* 84, 1410-1416 (1992)

58.H.J. Gabius HJ, C. Schröter , S. Gabius,, U. Brinck , L.F. Tietze: Binding of T-antigen-bearing neoglycoprotein and peanut agglutinin to cultured tumor cells and breast carcinomas. *J Histochem Cytochem*. 38,1625-31 (1990)

59. Jonathan M. Rhodes, Barry J. Campbell, and Lu-Gang Yu: Lectin-epithelial interactions in the human colon. *Biochem Soc Trans* 36, 1482-1486 (2008)

60. L. G. Yu, J. D. Milton, D. G. Fernig, and J. M. Rhodes: Opposite effects on human colon cancer cell proliferation of two dietary Thomsen-Friedenreich antigen-binding lectins. *J Cell Physiol* 186, 282-287 (2001)

61. B. J. Campbell, I. A. Finnie, E. F. Hounsell, and J. M. Rhodes: Direct demonstration of increased expression of Thomsen-Friedenreich (TF) antigen in colonic adenocarcinoma and ulcerative colitis mucin and its concealment in normal mucin. *J Clin Invest* 95, 571-576 (1995)

62. Katarina Dahlenborg, Lena Hultman, Roland Carlsson, and Bo Jansson: Human monoclonal antibodies specific for the tumour associated Thomsen-Friedenreich antigen. *International Journal of Cancer* 70, 63-71 (1997)

63.F. J. Irazoqui, B. Jansson, P. H. Lopez, and G. A. Nores: Correlative fine specificity of several Thomsen-Friedenreich disaccharide-binding proteins with an effect on tumor cell proliferation. *J. Biochem* 130, 33-37 (2001)

64.J. Bray, R. U. Lemieux, and T. A. McPherson: Use of a synthetic hapten in the demonstration of the Thomsen-Friedenreich (T) antigen on neuraminidase-treated human red blood cells and lymphocytes. *J Immunol* 126, 1966-1969 (1981)

65. Gunter Butschakand Uwe Karsten: Isolation and characterization of thomsen-friedenreich-specific antibodies from human serum. *Tumour Biol* 23, 113-122 (2002)

66. B. J. Campbell, I. A. Finnie, E. F. Hounsell, and J. M. Rhodes: Direct demonstration of increased expression of Thomsen-Friedenreich (TF) antigen in colonic adenocarcinoma and ulcerative colitis mucin and its concealment in normal mucin. *J Clin Invest* 95, 571-576 (1995)

67. Y. Cao, U. Karsten, G. Otto, and P. Bannasch: Expression of MUC1, Thomsen-Friedenreich antigen, Tn, sialosyl-Tn, and alpha2,6-linked sialic acid in hepatocellular carcinomas and preneoplastic hepatocellular lesions. *Virchows Arch* 434, 503-509 (1999)

68. Y. Cao, U. Karsten, H. Zerban, and P. Bannasch: Expression of MUC1, Thomsen-Friedenreich-related antigens, and cytokeratin 19 in human renal cell carcinomas and tubular clear cell lesions. *Virchows Arch* 436, 119-126 (2000)

69. Y. Cao, P. M. Schlag, and U. Karsten: Immunodetection of epithelial mucin (MUC1, MUC3) and mucin-associated glycotopes (TF, Tn, and sialosyl-Tn) in benign and malignant lesions of colonic epithelium: apolar localization corresponds to malignant transformation. *Virchows Arch* 431, 159-166 (1997)

70. K. Dahlenborg, L. Hultman, R. Carlsson, and B. Jansson: Human monoclonal antibodies specific for the tumour associated Thomsen-Friedenreich antigen. *Int J Cancer* 70, 63-71 (1997)

71. J. Samuel, A. A. Noujaim, G. D. MacLean, M. R. Suresh, and B. M. Longenecker: Analysis of human tumor associated Thomsen-Friedenreich antigen. *Cancer Res* 50, 4801-4808 (1990)

72. K. R. Diakun, S. Yazawa, L. Valenzuela, S. A. Abbas, and K. L. Matta: Synthetic antigens as immunogens: Part II: Antibodies to synthetic T antigen. *Immunological Investigations* 16, 151-163 (1987).

73. W. Dippold, A. Steinborn, and K. H. Meyer zum Buschenfelde: The role of the Thomsen-Friedenreich antigen as a tumor-associated molecule. *Environ Health Perspect* 88, 255-257 (1990).

74.C. J. Turner, T. R. Sykes, B. M. Longenecker, and A. A. Noujaim: Comparative radiolabeling and distribution of a tumour-directed monoclonal antibody. *Int J Rad Appl Instrum B* 15, 701-706 (1988)

75. S. A. McQuarrie, T. Riauka, R. P. Baum, T. R. Sykes, A. A. Noujaim, G. Boniface, G. D. MacLean, and A. J. McEwan: The effects of circulating antigen on the pharmacokinetics and radioimmunoscintigraphic properties of 99m Tc labelled monoclonal antibodies in cancer patients. *Journal of Pharmacy & Pharmaceutical Sciences* 1, 115-125 (1998)

76. B. M. Longenecker, D. J. Willans, G. D. MacLean, S. Selvaraj, M. R. Suresh, and A. A. Noujaim: Monoclonal antibodies and synthetic tumor-associated glycoconjugates in the study of the expression of Thomsen-Friedenreich-like and Tn-like antigens on human cancers. *J Natl Cancer Inst* 78, 489-496 (1987)

77. A. F. Rahmanand B. M. Longenecker: A monoclonal antibody specific for the Thomsen-Friedenreich cryptic T antigen. *J Immunol* 129, 2021-2024 (1982)

78.U. Karsten, G. Butschak, Y. Cao, S. Goletz, and F. G. Hanisch: A new monoclonal antibody (A78- G/A7) to the Thomsen-Friedenreich pan-tumor antigen. *Hybridoma* 14, 37-44 (1995)

79. K. Rittenhouse-Diakun, Z. Xia, D. Pickhardt, S. Morey, M. G. Baek, and R. Roy: Development and characterization of monoclonal antibody to T-antigen: (Gal beta1-3GalNAc-alpha-O). *Hybridoma* 17, 165-173 (1998)

80.L. G. Yu, B. Jansson, D. G. Fernig, J. D. Milton, J. A. Smith, O. V. Gerasimenko, M. Jones, and J. M. Rhodes: Stimulation of proliferation in human colon cancer cells by human monoclonal antibodies against the TF antigen (galactose beta1-3 N-acetyl-galactosamine). *Int J Cancer* 73, 424-431 (1997)

81. K. S. McCarty, Jr.: Anti-Thomsen-Friedenreich (T) antibody and its application to human breast carcinoma: where are we after 20 years? *J Immunol Methods* 188, 171-173 (1995)

82. Tongzhong Ju, Vivianne I. Otto, and Richard D. Cummings: The tn antigen-structural simplicity and biological complexity. *Angew Chem Int Ed Engl* 50, 1770-1791 (2011)

83.B. J. Campbell, G. E. Rowe, K. Leiper, and J. M. Rhodes: Increasing the intra-Golgi pH of cultured LS174T goblet-differentiated cells mimics the decreased mucin sulfation and increased Thomsen-Friedenreich antigen (Gal beta1-3GalNac alpha-) expression seen in colon cancer. *Glycobiology* 11, 385-393 (2001)

84. Lu-Gang Yu, Nigel Andrews, Qicheng Zhao, Daniel McKean, Jennifer F. Williams, Lucy J. Connor, Oleg V. Gerasimenko, John Hilkens, Jun Hirabayashi, Kenichi

Kasai, and Jonathan M. Rhodes: Galectin-3 interaction with Thomsen-Friedenreich disaccharide on cancer-associated MUC1 causes increased cancer cell endothelial adhesion. *J Biol Chem* 282, 773-781 (2007)

85. Peter Gassmann, Mi-Li Kang, Soeren T. Mees, and Joerg Haier: In vivo tumor cell adhesion in the pulmonary microvasculature is exclusively mediated by tumor cell--endothelial cell interaction. *BMC Cancer* 10, 177 (2010)

86.Senitiroh Hakomori: Glycosylation defining cancer malignancy: new wine in an old bottle. *Proceedings of the National Academy of Sciences of the United States of America* 99, 10231-10233 (2002)

87. J.M.Guthridge, AM Kaplan, DA Cohen:, Regulation of B cell:T cell interactions: potential involvement of an endogenous B cell sialidase. *Immunol Invest.* 23(6-7):393-411 (1994)

88. Tongzhong Ju, Richard D. Cummings, and William M. Canfield: Purification, characterization, and subunit structure of rat core 1 Beta1,3-galactosyltransferase. *Journal of Biological Chemistry* 277, 169-177 (2002)

89. K. Kumamoto, Y. Goto, K. Sekikawa, S. Takenoshita, N. Ishida, M. Kawakita, and R. Kannagi: Increased expression of UDP-galactose transporter messenger RNA in human colon cancer tissues and its implication in synthesis of Thomsen-Friedenreich antigen and sialyl Lewis A/X determinants. *Cancer Res* 61, 4620-4627 (2001)

90. Antti Rivinoja, Nina Kokkonen, Ilmo Kellokumpu, and Sakari Kellokumpu: Elevated Golgi pH in breast and colorectal cancer cells correlates with the expression of oncofetal carbohydrate T-antigen. *J Cell Physiol* 208, 167-174 (2006)

91.Tongzhong Ju and Richard D. Cummings: A unique molecular chaperone Cosmc required for activity of the mammalian core 1 beta 3-galactosyltransferase. *Proceedings of the National Academy of Sciences of the United States of America* 99, 16613-16618 (2002)

92. F. Santos-Silva, A. Fonseca, T. Caffrey, F. Carvalho, P. Mesquita, C. Reis, R. Almeida, L. David, and M. A. Hollingsworth: Thomsen-Friedenreich antigen expression in gastric carcinomas is associated with MUC1 mucin VNTR polymorphism. *Glycobiology* 15, 511-517 (2005)

93. Richard D. Cummings Adjit Varki, Jeffery D. Esko, Pamela Stanley, Carolyn Bertozzi, Gerald Hart, Marilynn E. Etzler (ed.). Essentials of Glycobiology*, Cold Spring Harbor Laboratory Press,* Cold Spring Harbor, NY (2008)

94. Pratima Adhikari, Kiran Baccawat-Sikder, Celestine Thomas, R. Ravishankar, A. Arockia Jeyaprakash, Vivek Sharma, Mamanamana Vijayan, Avadhesha Surolia: Mutational Analysis at Asn-41 in Peanut Agglutinin. *J. Biol. Chem 276*, 40734-40739 (2001)

95. Barbara Kossowska, Eliza Lamer-Zarawska, Mariusz Olczak, Iwona Katnik-Prastowska: Lectin from *Beauveria bassiana* mycelium recognizes Thomsen-Friedenreich antigen and related structures. *Comp. Biochem. Physiol., B 123*, 23-31 (1999)

96. Albert Wu, Shunji Suggi: Differential binding properties of GalNAc and/or gal specific lectins. In: The molecular immunology of complex carbohydrates. Eds: Albert Wu, L. Garry Adams, Plenum Press, NY (1988)

97. Albert Wu, June. Wu, Zhanguang Yang, Tanuja Singh, Irwin, Goldstein, Nathan Sharon: Differential contributions of recognition factors of two plant lectins-*Amaranthus caudatus* lectin and *Arachis hypogea* agglutinin, reacting with Thomsen-Friedenreich disaccharide. *Biochimie 90*, 1769-1780 (2008)

98. Fernando Irazoqui, Bo Jansson, Pablo Lopez, Gustavo Nores: Correlative fine specificity of several Thomsen-Friedenreich Disaccharide-binding proteins with an effect on tumor cell proliferation. *J Biochem 130*, 33-37 (2001)

99. Kovacic, N, Muthing, J., and Marusic, A: Immunohistochemical and Flow Cytometric Analysis of Glycosphingolipid expression in Mouse Lymphoid tissues. *Journal of Histochemistry and Cytochemistry* 48:1677-1689 (2000)

100. DeBentzmann S, DuPutit PR, Bajolet-Laudeinat O, et al:Asialo GM1 is a receptor for Pseudomonas aeruginosa Adherance to Regenerating Respiratory Epithelial Cells. *Infection and Immunity* 64:1582-1588 (1996)

101. Alec Shysh, Soon Eu, Antonie Noujaim, Mavanur Suresh, B. Michael Longenecker: In vivo localization of radioiodinated peanut lectin in a murine TA3/Ha mammary carcinoma model. *Eur J Nucl Med 10*, 68-74 (1985)

102. Richa Chaturvedi, Jamie Heimburg., Jun Yan, Stephen Koury, Munwwar Sujjad, hani H. Abdel Nabi, Kate Rittenhouse-Olson: Tumor immunolocalization using 124-Iodine labeled JAA-F11antibody to Thomsen Friedenreich alpha-linked antigen. *Applied Radiations and isotopes 66*, 278-287 (2008)

103. Miller, GM, Andres, ML and Gridley, DS: NK cell depletion results in accelerated tumor growth and attenuates the anti-tumor effect of total body irradiation. *International Journal of Oncology* 1585-1592 (2003)

104. Ravinder Singh, Sreedhar Subramanian, Jonathan Rhodes, Barry Campbell: Peanut lectin stimulates proliferation of colon cancer cells by interaction with glycosylated CD44v6 isoforms and consequential activation of c-Met and MAPK: functional implications for disease-associated glycosylation changes. *Glycobiology 16*, 594-601(2006)

105. Lu Gang-Yu, Jeremy Milton, David Fernig, Jonathan Rhodes: Opposite effects on human colon cancer cell proliferation of two dietary Thomsen-Friedenreich antigen-binding lectin. *Journal of Cellular Physiology 186*, 282-287 (2001)

106. C. R. Boland, Y. F. Chen., S. J. Rinderle: Use of the lectin from *Amaranthus caudatus* as a histochemical probe of proliferating colonic epithelial cells. *Cancer Res* 51, 657-665 (1991)

107. Lu-Gang Yu, David Fernig., Michael Whites, David Spillers, Paul Appleton, Richard Evans, Ian Grierson, John Smith, Helen Davies, Oleg Gerasimenko, Ole Petersen, Jeremy Milton, Jonathan Rhodes: Edible mushroom (*Agaricus bisporus)* lectin, which reversibly inhibits epithelial cell proliferation, blocks nuclear localization sequence-dependent nuclear protein import. *J. Biol Chem 274*, 4890-4899 (1999)

108. Jonathan Rhodes, B. C., Lu-gang Yu: Lectin-epithelial interactions in the human colon. *Biochemical Society Transactions 36* (2008)

109. Sanjeev K. Mahanta, M. V. Krishna Sastry., Avadhesha Surolia : Topography of the combining region of a Thomsen-Friedenreich-antigen-specific lectin jacalin (*Artocarpus integrifolia* agglutinin). *Biochem J 265*, 831-840 (1990).

110. Kabir, S: Jacalin: a jackfruit (*Artocarpus heterophyllus)* seed-derived lectin of versatile applications in immunobiological research. *J. Immunol Methods 212*, 193-211 (1998)

111. G.L.Hortin, B.L. Trimpe: Lectin affinity chromatography of proteins bearing O-linked oligosaccharides: application of jacalin agarose. *Anal Biochem*, 188, 271-277 (1990)

112.H. Ahmed, B. P. Chatterjee: Further characterization and immunochemical studies on the carbohydrate specificity of jackfruit (*Arthocarpus integrifolia*) lectin. *J Biol Chem 264,* 9365-9372. (1989)

113. Remani, P., Augustine, J., Vijayan, K.K., Ankathil, R., Vasudevan,D.M., Nair, K.M., Vijayakumar, T.: Jackfruit lectin binding pattern in benign and malignant lesions of the breast. *In vivo* 3, 275 (1989)

114. Vijayakumar, T., Augustine, J., Mathew, L., Aleykutty, M.A.,Nair, B.M., Remani, P., Nair, K.M: Tissue binding pattern of plant lectins of thyroid. *J. Exp. Pathol*. 6, 11.(1992)

115. Pillai, K.R., Remani, P., Kannan, S., Mathew, A., Sujatan, K., Vijayakumar, T., Nair, M.K., Jackfruit lectin specific glycoconjugate expression during the progression of cervical intraepithelial neoplasia: a study on exfoliated cells. *Diagn.Cytopathol.* 10, 342 (1994)

116. Ning Li, Moonhee Park, Minglang Zhao, Julio Hilario-Vargas, David Mclnnes, Phillip Prisayanh, Zhi Liu, Luis Diaz: The Thomsen Friedenreich antigen-binding lectin Jacalin interacts with Desmoglein -1 and abrogates the pathogenicity of Pemphigus Foliaceus autoantibodies in vivo. *J Invest Dermatol130*, 2773-2780 (2010)

117. Fernando Irazoqui, Miguel Vides, Gustavo Nores: Structural requirements of carbohydrates to bind *Agaricus bisporus* lectin. *Glycobiology 9*, 59-64 (1999)

118. Vishwanath Chachadi, Shashikala Inamdar, Lu-Gang Yu, Jonathan Rhodes, Bale Swamy : Exquisite binding specificity of *Sclerotium rolfsii* lectin toward TF-related O-linked mucin-type glycan. *Glycoconju J*. (2011)

119. Demetres Leonidas, Bale Swamy, George hatzopoulos, Sathisha Gonchigar, Vishwanath Chachadi, Shashikala Inamdar, Spyros, Zographos, Nikos Oikonomakos : Structural basis for the carbohydrate recognition of the *Sclerotium rolfsii* Lectin. *J. Mol Biol 368*, 1145-1161 (2007)

120. Lei Feng, Hui Sun, ying Zhang, De-Feng Li, De-Cheng Wang: Structural insights into the recognition mechanism between an antitumor galectin AAL and the Thomsen-Friedenreich antigen. *The FASEB journal 24*, 3861-3868 (2010)

121. Luminita Damian, Didier Fournier., Mathias Winterhalter, Laurent Paquereau: Determination of thermodynamic parametersof Xerocomus chrysenteron lectin interaction with N-acetylgalactosamine and Thomsen Friedenreich antigen by isothermal titration calorimetry. *BMC Biochemistry 6* (2005)

122. Haike Ghazarian, Brian Idoni, and Steven B. Oppenheimer: A glycobiology review: Carbohydrates, lectins and implications in cancer therapeutics. *Acta Histochemica* 113, 236-247 (2011)

123. Lugang Yu, David Fernig, John Smith: Reversible inhibition of proliferation of epithelial cell lines by *Agaricus bisporus* (Edible Mushroom) lectin. *Cancer Res* 53, 4637-4632 (1993)

124. M.V. Swamy, P. B., S.R..Patanjali, M.J..Swamy, G.V..Swaralatha, A.Surolia: Analysis of saccharide binding to Artocarpus integrifolia lectin reveals specific recognition of T-antigen (beta-D-Gal(1----3)D-GalNAc). *J Biol Chem 261*, 11726-11733
(1986)

125. R. Stein, S. Chen, W. Grossman, and D. M. Goldenberg: Human lung carcinoma monoclonal antibody specific for the Thomsen-Friedenreich antigen. *Cancer Res* 49, 32-37 (1989)

126. O'Donnell RT, DeNardo SJ, Yuan A: Radioimmunotherapy with 111In/90Y-2IT-BAD-m170 for metastatic prostate cancer. Clin Cancer Res 2001;7:1561-8(2001)

127. Klaamas, K., Kurtenkov, O., Rittenhouse-Olson, K., Brjalin, V., Miljukhina, L., Shljapnikova, L., Engstrand, L: Expression of tumor-associated Thomsen-Friedenreich

Antigen (T Ag) in Helicobacter pylori and modulation of T Ag specific immune response in infected individuals. *Immunol Invest* 31, 191-204 (2002)

128. Kodar, K., O. Kurtenkov, and K. Klaamas: The Thomsen-Friedenreich antigen and alpha gal-specific human IgG glycoforms: concanavalin a reactivity and relation to survival of cancer patients. *Immunol Invest* 38, 704-17 (2009)

129. O. Kurtenkov, L. Miljukhina, J. Smorodin, K. Klaamas, N. Bovin, M. Ellamaa, and V. Chuzmarov: Natural IgM and IgG antibodies to Thomsen-Friedenreich (T) antigen in serum of patients with gastric cancer and blood donors--relation to Lewis (a,b) histo-blood group phenotype. *Acta Oncol* 38, 939-943 (1999)

130. O. Kurtenkov, K. Klaamas, S. Mensdorff-Pouilly, L. Miljukhina, L. Shljapnikova, and V. Chuzmarov: Humoral immune response to MUC1 and to the Thomsen-Friedenreich (TF) glycotope in patients with gastric cancer: relation to survival. *Acta Oncol* 46, 316-323 (2007)

131.E. P. Smorodin, O. A. Kurtenkov, B. L. Sergeyev, V. I. Chuzmarov, and V. P. Afanasyev: The relation of serum anti-(GalNAc beta) and -para-Forssman disaccharide IgG levels to the progression and histological grading of gastrointestinal cancer. *Exp Oncol* 29, 61-66 (2007)

132. O. Kurtenkov, K. Klaamas, K. Rittenhouse-Olson, L. Vahter, B. Sergejev, L. Miljukhina, and L. Shljapnikova: IgG immune response to tumor-associated carbohydrate antigens (TF, Tn, alphaGal) in patients with breast cancer: impact of neoadjuvant chemotherapy and relation to the survival. *Exp Oncol* 27, 136-140 (2005)

133. V. V. Glinsky, G. V. Glinsky, K. Rittenhouse-Olson, M. E. Huflejt, O. V. Glinskii, S. L. Deutscher, and T. P. Quinn: The role of Thomsen-Friedenreich antigen in adhesion of human breast and prostate cancer cells to the endothelium. *Cancer Res* 61, 4851-4857 (2001)

134. I. Takanami: Expression of Thomsen-Friedenreich antigen as a marker of poor prognosis in pulmonary adenocarcinoma. *Oncol Rep* 6, 341-344 (1999)

135. M. F. Wolf, A. Ludwig, P. Fritz, and K. Schumacher: Increased expression of Thomsen-Friedenreich antigens during tumor progression in breast cancer patients. *Tumour Biol* 9, 190-194 (1988)

136. Christian Schindlbeck, Udo Jeschke, Sandra Schulze, Uwe Karsten, Wolfgang Janni, Brigitte Rack, Harald Sommer, and Klaus Friese: Characterisation of disseminated tumor cells in the bone marrow of breast cancer patients by the Thomsen-Friedenreich tumor antigen. *Histochem Cell Biol* 123, 631-637 (2005)

137. H. Moriyama, H. Nakano, M. Igawa, and H. Nihira: T antigen expression in benign hyperplasia and adenocarcinoma of the prostate. *Urol Int* 42, 120-123 (1987)

138. Jeffrey E Lehr Kenneth J Pienta.: Preferential Adhesion of Prostate Cancer Cells to a Human Bone Marrow Endothelial Cell Line. *Journal of the National Cancer Institute* 90, 118-123 (1998)

139. H. Shigeoka, U. Karsten, K. Okuno, and M. Yasutomi: Inhibition of liver metastases from neuraminidase-treated colon 26 cells by an anti-Thomsen-Friedenreich-specific monoclonal antibody. *Tumour Biol* 20, 139-146 (1999)

140. Jamie Heimburg, Jun Yan, Susan Morey, Olga V. Glinskii, Virginia H. Huxley, Linda Wild, Robert Klick, Rene Roy, Vladislav V. Glinsky, and Kate Rittenhouse-Olson: Inhibition of spontaneous breast cancer metastasis by anti-Thomsen-Friedenreich antigen monoclonal antibody JAA-F11. *Neoplasia* 8, 939-948 (2006)

141. E. N. Peletskaya, V. V. Glinsky, G. V. Glinsky, S. L. Deutscher, and T. P. Quinn: Characterization of peptides that bind the tumor-associated Thomsen-Friedenreich antigen selected from bacteriophage display libraries. *J Mol Biol* 270, 374-384 (1997)

142. V. V. Glinsky, M. E. Huflejt, G. V. Glinsky, S. L. Deutscher, and T. P. Quinn: Effects of Thomsen-Friedenreich antigen-specific peptide P-30 on beta-galactoside-mediated homotypic aggregation and adhesion to the endothelium of MDA-MB-435 human breast carcinoma cells. *Cancer Res* 60, 2584-2588 (2000)

143. Vladislav V. Glinsky, Gennadi V. Glinsky, Olga V. Glinskii, Virginia H. Huxley, James R. Turk, Valeri V. Mossine, Susan L. Deutscher, Kenneth J. Pienta, and Thomas P. Quinn: Intravascular metastatic cancer cell homotypic aggregation at the sites of primary attachment to the endothelium. *Cancer Res* 63, 3805-3811 (2003)

144. Jun Zou, Vladislav V. Glinsky, Linda A. Landon, Leslie Matthews, and Susan L. Deutscher: Peptides specific to the galectin-3 carbohydrate recognition domain inhibit metastasis-associated cancer cell adhesion. *Carcinogenesis* 26, 309-318 (2005)

145. Khaldoyanidi, S. K., Glinsky, V. V., Sikora, L., Glinskii, A. B., Mossine, V. V., Quinn, T. P., Glinsky, G. V., and Sriramarao, P.: MDA-MB-435 human breast carcinoma cell homo- and heterotypic adhesion under flow conditions is mediated in part by Thomsen-Friedenreich antigen-galectin-3 interactions. *J Biol Chem 278*, 4127-4134 (2003)

146. Olga V. Glinskii, James R. Turk, Kenneth J. Pienta, Virginia H. Huxley, and Vladislav V. Glinsky: Evidence of porcine and human endothelium activation by cancer-associated carbohydrates expressed on glycoproteins and tumour cells. *J Physiol* 554, 89-99 (2004)

147. Olga V. Glinskii, Virginia H. Huxley, Gennadi V. Glinsky, Kenneth J. Pienta, Avraham Raz, and Vladislav V. Glinsky: Mechanical entrapment is insufficient and intercellular adhesion is essential for metastatic cell arrest in distant organs. *Neoplasia* 7, 522-527 (2005)

148. Qicheng Zhao, Monica Barclay, John Hilkens, Xiuli Guo, Hannah Barrow, Jonathan M. Rhodes, and Lu-Gang Yu: Interaction between circulating galectin-3 and cancer-associated MUC1 enhances tumour cell homotypic aggregation and prevents anoikis. *Mol Cancer* 9, 154 (2010)

149. R. Singh, B. J. Campbell, L. G. Yu, D. G. Fernig, J. D. Milton, R. A. Goodlad, A. J. FitzGerald, and J. M. Rhodes: Cell surface-expressed Thomsen-Friedenreich antigen in colon cancer is predominantly carried on high molecular weight splice variants of CD44. *Glycobiology* 11, 587-592 (2001)

150. A. M. Havens, Y. Jung, Y. X. Sun, J. Wang, R. B. Shah, H. J. Buhring, K. J. Pienta, and R. S. Taichman: The role of sialomucin CD164 (MGC-24v or endolyn) in prostate cancer metastasis. *BMC Cancer* 6, 195 (2006)

151. Alaa M. Afify, Stacey Tate, Blythe Durbin-Johnson, David M. Rocke, and Thomas Konia: Expression of CD44s and CD44v6 in lung cancer and their correlation with prognostic factors. *Int J Biol Markers* 26, 50-57 (2011)

152. Hiroko Nogi, Masafumi Suzuki, Makiko Kamio, Kumiko Kato, Kazumi Kawase, Yasuo Toriumi, Hiroshi Takeyama, Hisaki Fukushima, Toshiaki Morikawa, and Ken Uchida: Impact of CD44+CD24- cells on non-sentinel axillary lymph node metastases in sentinel node-positive breast cancer. *Oncol Rep* 25, 1109-1115 (2011)

153. Nobuhiro Uwa, Tatsuki R. Kataoka, Ikuko Torii, Ayuko Sato, Takashi Nishigami, Misa Song, Takashi Daimon, Nobuo Saeki, Kousuke Sagawa, Takeshi Mouri, Tomonori Terada, Masafumi Sakagami, and Tohru Tsujimura: CD44 expression is related to poor prognosis of hypopharyngeal squamous cell carcinoma. *Acta Otolaryngol* 131, 323-329 (2011)

154. Kai-Hung Wang, An-Pei Kao, Chia-Cheng Chang, Jau-Nan Lee, Ming-Feng Hou, Cheng-Yu Long, Hung-Sheng Chen, and Eing-Mei Tsai: Increasing CD44+/CD24(-) tumor stem cells, and upregulation of COX-2 and HDAC6, as major functions of HER2 in breast tumorigenesis. *Mol Cancer* 9, 288 (2010)

155. Steffen Goletz, Yi Cao, Antje Danielcyk, Peter Ravn, Schoeber U, and Uwe Karsten: Thomsen-Friedenreich antigen: the 'hidden' tumor antigen. *Adv Exp Med Biol* 535, 147-162 (2003)

156. Alessandra Franco: CTL-based cancer preventive/therapeutic vaccines for carcinomas; role of tumour-associated carbohydrate antigens. *Scand J Immunol* 61, 391-397 (2005)

157. Georg F. Springer, Parimal R. Desai, Herta Tegtmeyer, Bruce D. Spencer, and Edward F. Scanlon: Pancarcinoma T/Tn antigen detects human carcinoma long before biopsy does and its vaccine prevents breast carcinoma recurrence. *Ann NY Acad Sci* 690, 355-357 (1993)

158. Peter Y. S. Fung, Marian Madej, R. Rao Koganty, and B. Michael Longenecker: Active specific immunotherapy of a murine mammary adenocarcinoma using a synthetic tumor-associated glycoconjugate. *Cancer Res* 50, 4308-4314 (1990)

159. Grant D. MacLean, Mary Beth Bowen-Yacyshyn, Samuel J, Allison Meikle, Gavin Stuart, Jill Nation, Sibrand Poppema, Jerry M, R. Rao Koganty, Wong T, and B. Michael Longenecker: Active immunization of human ovarian cancer patients against a common carcinoma (Thomsen-Friedenreich) determinant using a synthetic carbohydrate antigen. *J Immunotherapy* 11(4), 292-305 (1992)

160. Yanfei Xu, Sandra J. Gendler, and Alessandra Franco: Designer glycopeptides for CTL-based elimination of carcinomas. *J Exp Med* 199, 707-716 (2004)

161. Sucharita Adluri, Friedhelm Helling, Shunichiro Ogata et al: Immunogenicity of synthetic TF-KLH (keyhole limpet hemocyanin) and sTn-KLH conjugates in colorectal carcinoma patients. *Cancer Immunol Immunother* 41, 185-192 (1995)

162. Susan F. Slovin S, Govindaswami Ragupathi, Cristina Musseli, Celina Fernandez, Meghan Diani, David Verbal, Samuel J. Danishefsky, Philip O. Livingston, and Howard I. Scher: Thomsen-Friedenreich (TF) antigen as a target for prostate cancer vaccine: clinical trial results with TF cluster (c)-KLH plus QS21 conjugate vaccine in patients with biochemically relapsed prostate cancer. *Cancer Immunol Immunother* 54 (7), 694-702 (2005)

163. Charlotte R. Kensil, Usha Patel, Michael Lennick, and Dante Marciani: Separation and characterization of saponins with adjuvant activity from Quillaja saponaria Molina cortex. *J Immunol* 146, 431–437(1991)

164. Hong-Xiang Suna, Yong Xiea, and Yi-Ping Yec: Advances in saponin-based adjuvants. *Vaccine* 27, 1787–1796 (2009)

165. Soo Kie Kim, Govindaswami Ragupathi, Sarah Cappello, Ella Kagan, and Philip O Livingston: Comparison of the effect of different immunological adjuvants on the antibody and T-cell response to immunization with MUC1-KLH and GD3-KLH conjugate vaccines. *Vaccine* 18, 597-603. (2000)

166. Soo Kie Kim1, Govindaswami Ragupathi, Sarah Cappello, Ella Kagan, and Philip O. Livingston: Effect of immunological adjuvant combinations on the antibody and T-cell response to vaccination with MUC1-KLH and GD3- KLH conjugates. Vaccine 19, 530-537 (2001)

167. Susan F. Slovin S, Govindaswami Ragupathi, Celin Fernandez et al: A polyvalent vaccine for high-risk prostate patients: 'are more antigens better?' *Cancer Immunol Immunother* 56, 1921-1930 (2007)

168. Paul J. Sabbatini, Govindaswami Ragupathi, Chandra Hood et al: Pilot study of a heptavalent vaccine-keyhole limpet hemocyanin conjugate plus QS-21 in patients with

epithelial ovarian, fallopian tube, or peritoneal cancer. *Clin Cancer Res* 13, 4170 (2007)

169. Anja Hoffmann-Röder, Anton Kaiser, Sarah Wagner, Nikola Gaidzik, Danuta Kowalczyk, Ulrika Westerlind, Bastian Gerlitzki, Edgar Schmitt and Horst Kunz: Synthetic Antitumor Vaccines from Tetanus Toxoid Conjugates of MUC1 Glycopeptides with the Thomsen–Friedenreich Antigen and a Fluorine-Substituted Analogue. *Angew Chem Int Ed* 49, 8498-8503 (2010)

170. Victor G. Sendra, Natacha Zlocowski, Yanina Ditamo, Silvina Copioli, Mads P. Tarp, Eric P. Benett, Henrik Clausen, German A. Roth, Gustavo A. Nores, and Fernando J. Irazoqui: Glycan bioengineering in immunogen design for tumor T antigen immunotargeting. *Mol Immunol* 46, 3445-3453 (2009)

171. Sampat Ingale, Margreet A. Wolfert, Jidnyasa Gaekwad, Theresa Buskas, and Geert-Jan Boons: Robuse immune responses elicited by a fully synthetic three-component vaccine. *Nat. Chem. Biol* 3, 663-667 (2007)

172. Brendan L. Wilkinson, Stephanie Day, Lara R. Malins, Vasso Apostolopoulos, and Richard J. Payne: Self-Adjuvanting Multicomponent Cancer Vaccine Candidates Combining Per-Glycosylated MUC1 Glycopeptides and the Toll-like Receptor 2 Agonist $Pam_3CysSer$. *Angew Chem Int Ed* 50, 1635–1639 (2011).

173. Behjatolah Monzavi-Karbassi and Thomas Kieber Emmons: Current concepts in cancer vaccine strategies. *Biotechniques* 30, 170-189 (2001)

174. Melita B. Irving, Oscar Pan, and Jamie K. Scott: Random-peptide libraries and antigen fragment libraries for epitope mapping and the development of vaccines and diagnostics. *Curr Opin Chem Biol* 5, 314–324 (2001)

175. Behjatolah Monzavi-Karbassi, Gina Cunto-Amesty, Ping Luo, and Thomas Kieber-Emmons: Peptide mimotopes as surrogate antigens of carbohydrates in vaccine discovery. *Trends Biotechnol* 20, 207–214 (2002)

176. Seth H. Pincus, Michael J. Smith, Harold J. Jennings, James B. Burritt, and Pati M. Glee: Peptides that mimic the group B streptococcal type III capsular polysaccharide antigen. *J Immunol* 160;293-298 (1998)

177. Ping Luo, Michael Agadjanyan, Jianping Qiu, M.A. Julie Westerrink, Zenon Steplewski, and Thomas Kieber-Emmons: Antigenic and immunological mimicry of peptide mimotopes of Lewis carbohydrate antigen. *Mol Immunol* 35, 865-879 (1998)

178. Britta Hardy and Annat Raiter: A mimotope peptide-based anti-cancer vaccine selected by BAT monoclonal antibody. Vaccine 23, 4283-4291 (2005)

179. Stephen Wagner, Christine Hafner, Dorothee Allwardt D et al: Vaccination with a human high molecular weight melanoma-associated antigen mimotope induces a humoral response inhibiting melanoma cell growth in vitro. *J Immunology* 174: 976-982 (2005)

180. Jamie Heimburg-Molinaro, Adel Almogren, Susan Morey, Olga V Glinskii, Rene Roy, Gregory E. Wilding, Richard P. Cheng, Vladislav V. Glinsky, and Kate Rittenhouse-Olson: Development, Characterization, and Immunotherapeutic Use of Peptide Mimics of the Thomsen-Friedenreich Carbohydrate Antigen. *Neoplasia* 11, 780–792 (2009)

181. Dennis M. Klinman: Immunotherapeutics uses of CpG Oligodeoxynucleotides: *Nat Rev Immunol* 4, 1-10 (2004)

182. Luigi Aurisicchio, Daniela Peruzzi, Antonella Conforti et al: Treatment of mammary carcinomas in HER-2 transgenic mice through combination of genetic vaccine and an agonist of toll-like receptor 9. *Clin Cancer Res* 15(5) 1575-1584 (2009)

183. Anke R. Kraft, Frank Krux, Simone Schimmer, Claes Ohlen, Philip D. Greenberg and Ulf Dittmer: CpG Oligodeoxynucleotides allow for effective adoptive therapy in chronic retroviral infection. *Blood* 109(7), 2982-2984 (2007)

184. Umer J. Chaudry, T. Peter Kigham, Georg Plitas, Steven C. Katz, Jesse R. Raub, and Ronald P. DeMatteo: Combined stimulation with interleukin-18 and CpG induces murine natural killer dendrtic cells to produce IFN-gamma and inhibit tumor growth. *Cancer Res* 66 (21), 10497-10504 (2006)

185. Arthur M. Krieg: Toll-like receptor 9 (TLR9) agonists in the treatment of cancer. *Oncogene* 27; 161-167 (2008)

186. Jorg Vollmer and Arthur M. Krieg: Immunotherapeutic applications of CpG oligonucleotides TLR9 agonists. *Adv Drug Delivery Rev* 61, 195-204 (2009)

187. Johanna E. Portielje, Jan Gratama, Heidi H. van Ojik, Gerrit Stoter and Wim H. Kruit: IL-12: a promising adjuvant for cancer vaccination. *Cancer Immunol Immunother* 23, 307-313 (2003)

188. Mohamed L. Salem, Andre N. Kadima, Yuehua Zhou et al: Paracrine release of IL-12 stimulates IFN-gamma production and dramatically enhances the antigen-specific T Cell response after vaccination with a novel peptide-based cancer vaccine. *J Immunol* 172(9), 5159-5167 (2004)

189. Paul W. Dempsey, Michael E.D. Allison, Srinivas Akkaraju, Christopher C. Goodnow, and Douglas T. Fearon: C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. *Science*271, 348–356 (1996)

190. Thomas D. Green, David C. Montefiori, and Ted M. Ross: Enhancement of antibodies to the human immunodeficiency virus type 1 envelope by using the molecular adjuvant C3d. *J Virol* 77(3), 2046–2055 (2003)

191. Pei-he Liang, Ke-qin Zhang, Gui-Lian Xu, Yan-feng Li, Luo-fu Wang, Zhi-lin Nie, Jin Ye-Gang Wu, Cheng-guo Ge and Feng-shuo Jin: Construction of a DNA vaccine encoding Flk-1 extracellular domain and C3d fusion gene and investigation of its suppressing effect on tumor growth. *Cancer Immunol Immunother* 59(1), 93–101 (2010)

192. Andreas Sundgren and Joseph J. Barchi, Jr.: Varied presentation of the Thomsen-Friedenreich disaccharide tumor-associated carbohydrate antigen on gold nanoparticles. *Carbohydr Res* 343, 1594-1604 (2008)

193. Antoinette Hollestelle and Mieke Schutte: Comment Re: MDA-MB-435and M14 Cell Lines: Identical but not M14 Melanoma? Cancer Res 2009; 69: (19) (2009)

194. Ann F. Chambers: MDA-MB-435 and M14 Cell Lines: Identical but not M14 Melanoma? Cancer Res 2009; 69: (13). (2009)

Key Words: Thomsen-Friedenreich antigen, TF-Ag, Metastasis, Vaccine, Active Immunotherapy, Passive Immunotherapy, Review

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