Predictive Biomarkers and Personalized Medicine

Serum Antibodies to Blood Group A Predict Survival on PROSTVAC-VF

Christopher T. Campbell1, James L. Gulley2, Oyindasola Oyelaran1, James W. Hodge2, Jeffrey Schlom2, and Jeffrey C. Gildersleeve1

Abstract

Purpose: There is evidence that therapeutic cancer vaccines can lengthen survival for some patients with cancer, but responses vary widely from one person to another. Methods to predict clinical outcomes will advance the field and provide new insights into critical determinants of in vivo efficacy.

Experimental Design: This retrospective study included 141 subjects from phase II trials of PROST-VAC-VF, a poxvirus-based cancer vaccine currently in phase III clinical trials for advanced prostate cancer. A glycan microarray was used to profile prevaccination antiglycan antibody populations in sera as potential biomarkers for PROSTVAC-VF. The screen for predictive biomarkers identified antiglycan antibodies that consistently stratified subjects into groups with different Kaplan–Meier survival estimates. Because of the potential for overfitting, a permutation test was used to estimate the false discovery rate.

Results: Prevaccination antibody levels to blood group A trisaccharide (BG-Atri) were found to have a statistically significant correlation with survival. Long-term survival was approximately doubled in subjects with abundant anti-BG-Atri immunoglobulin M (IgM) relative to subjects with little or no preexisting IgM for BG-Atri. This survival correlation was specific to vaccine treatment, as no correlation was observed in control patients immunized with wild-type poxviruses lacking the key tumor antigen, prostate-specific antigen (PSA). Moreover, anti-BG-Atri IgM levels were not correlated with general measures of disease severity, such as PSA levels, Gleason score, or Halabi predicted survival.

Conclusion: In addition to reporting a new potentially predictive biomarker for PROSTVAC-VF, this study highlights the use of glycan microarray technology for improving our understanding of vaccine immunology.

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Introduction

Personalized medicine bases treatment decisions on characteristics of individual patients and their diseases (1). One particular area set to benefit from personalized medicine is cancer vaccine therapy (2), which trains the immune system to recognize and eliminate malignant cells. While numerous clinical trials have been conducted and one therapeutic cancer vaccine has earned U.S. Food and Drug Administration (FDA) approval, clinical responses vary widely from one patient to another. Certain patients achieve long-lasting responses, whereas others seem to receive little or no benefit. A longstanding objective in the field is to devise methods to predict a patient's benefit from cancer vaccine therapy. By targeting treatment to likely responders, a predictive method can significantly improve clinical efficacy while simultaneously reducing health care costs.

Methods to optimize treatment decisions generally fall into 2 categories: (i) assessing immune responses after vaccination, and (ii) predicting clinical outcomes before vaccination. A number of studies have identified survival associations with immune responses measured after vaccination. Potential biomarkers (reviewed in refs. 3–5) include T-cell responses, delayed type hypersensitivity, immunosuppressive regulatory T cells, cytokine profiles, humoral responses, antivector titers, and circulating tumor cells. While promising, none of these approaches have been fully validated. Moreover, these methods require treatment of patients for at least several months before assessing response. Methods to predict outcomes before treatment can avoid unnecessary side effects in patients unlikely to benefit from the vaccine as well as allow patients to pursue alternative treatments at an earlier time point in the course of their disease. At present,
Recruiting patients for cancer vaccine trials is challenging, as high response rates are needed to demonstrate clinical benefit. Preexisting antibodies to vaccine components could affect the immune response to vaccination, thus leading to variable clinical outcomes. For example, patients with preexisting antibodies against tumor antigens may have attenuated vaccine-induced responses, whereas patients with low or absent preexisting antibodies may be more likely to respond to vaccination. Therefore, identifying biomarkers that can predict vaccine response is critical to optimizing treatment outcomes.

Several studies have explored the correlation between preexisting antibodies and clinical outcomes in cancer vaccine trials. In a study by Campbell et al., serum antiglycan antibodies were found to be associated with improved survival in patients treated with a cancer vaccine. These antibodies were prevalent in a large proportion of the study population and were independent of various clinical and pathological factors. The authors propose that serum antiglycan antibodies may be a useful biomarker for predicting vaccine efficacy.

Methods

We used glycan microarrays as part of a glycoimmunomics strategy (high-throughput profiling of serum anti-carbohydrate antibodies) to evaluate whether preexisting serum antiglycan antibodies correlate with clinical response to PROSTVAC-VF, a poxvirus-based therapeutic cancer vaccine in phase III clinical trials for the treatment of advanced prostate cancer. PROSTVAC-VF induces immunity for prostate-specific antigen (PSA) using genetically modified vaccinia and fowlpox encoding PSA and 3 costimulatory molecules (CD40, ICAM-1, and B7.1) coadministered with granulocyte macrophage colony-stimulating factor (GM-CSF). In 2 phase II clinical trials (23, 24), PROSTVAC-VF treatment was associated with preliminary evidence of a 8 to 9 months improvement in median overall survival. While PROSTVAC-VF shows promise and has entered a phase III trial (25), not all patients experience improved survival. Because PROSTVAC-VF uses viral vectors that are glycosylated, generates responses to a glycoprotein (PSA), and stimulates immunity to tumors that display tumor-associated carbohydrate antigens, we hypothesized that preexisting antiglycan antibody levels influence clinical outcomes.

In this study, we show that survival outcomes for PROSTVAC-VF correlate with humoral immunity for a blood group A–like glycan found on its viral vectors. These antibodies provide a promising emerging biomarker for predicting an individual patient’s response to PROSTVAC-VF. In addition, these results suggest antiglycan antibodies are an undereexplored reservoir of biomarkers for personalized medicine.

Patients and Methods

Serum samples

Sera from PROSTVAC-VF recipients (n = 28 for training; n = 76 for validation) and controls (n = 37) came from phase II clinical trials (23, 24). Sera were stored at −70°C. Clinical data were blinded during data collection and processing.

Translational Relevance

Cancer vaccines can be very effective for treating patients, but clinical responses vary widely from one patient to another. Strategies to target treatment to likely responders could significantly improve clinical efficacy, minimize unnecessary side effects, and reduce health care costs. In this study, we show that overall postvaccination survival for patients treated with PROSTVAC-VF correlates with preexisting serum immunoglobulin M levels for the blood group A glycan. Antibody group A levels did not correlate with survival in control patients and were independent of various measures of disease severity. Therefore, measurement of serum antibody group A levels before vaccination could provide a simple method for preselecting patients that are likely to benefit from PROSTVAC-VF therapy. Finally, these studies emphasize the potential of serum antiglycan antibodies as a convenient, understudied reservoir of biomarkers to advance personalized medicine and suggest new avenues for improving poxvirus-based vaccines.

However, the factors that predispose an individual to have a productive response to a vaccine, thus leading to improved survival, are unknown.

Preexisting antibodies potentially influence clinical outcomes of patients treated with a cancer vaccine in several ways. Numerous antitumor autoantibodies have been reported (6, 7), and these antibodies may induce antibody-dependent cell-mediated cytotoxicity, block metastasis, or directly kill tumors. These antibodies could act synergistically with vaccine-induced responses leading to improved survival. In addition, preexisting humoral immunity may augment vaccine responses by enhancing cross-presentation (8) and antigen spreading (9). Alternatively, other preexisting antibodies may promote tumor growth antagonizing vaccine-induced responses. Fourth, preexisting antibodies to vaccine components could affect immune responses induced by vaccination (10). While preexisting antibodies could influence vaccine efficacy in many ways, most studies have shown no correlation, and the few reported correlations were modest trends toward improved survival or disease stabilization in a small number of subjects (11, 12).

Prior studies on relationships between serum antibodies and survival have primarily focused on antibodies that recognize proteins and/or peptides (13, 14). Carbohydrates are an equally important class of antigens, and many cancer vaccines currently in clinical trials, such as viral vector vaccines, whole-cell vaccines, glycoprotein-based vaccines, and carbohydrate-based vaccines, possess carbohydrates that could be targeted by the immune system. Abnormal glycosylation is a hallmark of malignancy (15, 16), and many of the glycans on these vaccines, as well as the tumors being treated, are considered tumor-associated antigens. Antibodies that recognize tumor-associated carbohydrate antigens have frequently been observed in patients with cancer. Certain antiglycan antibodies have been shown to be involved in tumor immunosurveillance (17) and regulating autoimmunity (18), and in some cases, antiglycan antibodies have shown promise for early detection and prognosis of cancer (e.g., refs. 19, 20). Much less, however, is known about this family of antibodies as treatment-specific biomarkers.

While many antiglycan antibodies may contribute to clinical outcome, a priori prediction of the most relevant glycans remains difficult. Technical limitations prevent the characterization of any virus or organism’s glycome and conventional assays for immunogenic properties of individual glycans remain arduous. Glycan microarrays (21, 22), which contain hundreds of carbohydrates immobilized on microscope slides, overcome many of these technical limitations by providing a multiplex assay for profiling serum antiglycan antibodies requiring only minute amounts of available glycans and clinical samples.

We used glycan microarrays as part of a glycoimmunomics strategy (high-throughput profiling of serum anti-carbohydrate antibodies) to evaluate whether prevaccination levels of serum antiglycan antibodies correlate with clinical response to PROSTVAC-VF, a poxvirus-based therapeutic cancer vaccine in phase III clinical trials for the treatment of advanced prostate cancer. PROSTVAC-VF induces immunity for prostate-specific antigen (PSA) using genetically modified vaccinia and fowlpox encoding PSA and 3 costimulatory molecules (LEA-3, B7.1, and ICAM-1) coadministered with granulocyte macrophage colony-stimulating factor (GM-CSF). In 2 phase II clinical trials (23, 24), PROSTVAC-VF treatment was associated with preliminary evidence of a 8 to 9 months improvement in median overall survival. While PROSTVAC-VF shows promise and has entered a phase III trial (25), not all patients experience improved survival. Because PROSTVAC-VF uses viral vectors that are glycosylated, generates responses to a glycoprotein (PSA), and stimulates immunity to tumors that display tumor-associated carbohydrate antigens, we hypothesized that preexisting antiglycan antibody levels influence clinical outcomes.

In this study, we show that survival outcomes for PROSTVAC-VF correlate with humoral immunity for a blood group A–like glycan found on its viral vectors. These antibodies provide a promising emerging biomarker for predicting an individual patient’s response to PROSTVAC-VF. In addition, these results suggest antiglycan antibodies are an undereexplored reservoir of biomarkers for personalized medicine.
High-throughput profiling of serum antiglycan antibodies

Serum antiglycan antibodies (diluted 1:50 and 1:200) were profiled on a glycan microarray (204 array components listed in Supplementary Table S1), which previously has been validated with numerous monoclonal antibodies and lectins (26–28) and shown to have excellent reproducibility (29). Arrays were printed as previously described (30, 31) except SMP2 pins (TeleChem) replaced SMP3 pins, and the print buffer included DyLight 649 (0.7 μg/mL; Thermo Scientific) to assess print quality before being washed away before serum assays. Bound antiglycan antibodies were detected with fluorescent secondary antibodies (Jackson ImmunoResearch) specific for IgM, immunoglobulin G (IgG), or total immunoglobulin.

Because more than 800 arrays were required, experiments were designed to minimize technical variations and help assess quality (32). Slides came from the fewest possible print batches, and consistency of print batches was checked using reference serum. Samples were analyzed in a random order to ensure intermixing of controls and vaccinated subjects with varied responses to PROSTVAC-VF. In addition, the same experimenter collected all array data, and samples were analyzed on replicate slides to identify technical faults.

The data discussed in this publication have been deposited in National Center for Biotechnology Information’s (NCBI) Gene Expression Omnibus (GEO; 33) and are accessible through GEO Series accession number GSE42184 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=hzafetseeemif&acc=GSE42184).

Glycan profiling of viral vectors

Glycans on viral vectors were assayed by adapting the glycan microarray for a competitive binding assay. Serum [diluted 1:500 in 1% bovine serum albumin (BSA) and 0.3% human serum albumin] was preblocked for 1.5 hours at 37°C with vaccinia (9.1E9 pfu) and fowlpox (6.9E10 pfu) that had been propagated in chicken embryo dermal cells, purified with a sucrose gradient, and UV inactivated. Presence of particular glycans on viral vectors was detected as control serum. Reduced binding of preblocked serum relative to binding of total immunoglobulin.

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Second, ELISA measured BG-A glycans on viral vectors. Viral vectors (diluted to 10 μg/mL in carbonate buffer, pH 9.6) were coated onto Maxisorp plates (Nunc) and incubated with monoclonal anti-BG-A antibody HE-195 (Genex; diluted 1:100 in PBS + 3% BSA). Next, bound monoclonal antibody was detected with alkaline phosphatase–conjugated secondary antibody (Jackson ImmunoResearch; diluted 1:250) that catalyzed the conversion of methylumbelliferylphosphate (Sigma; 26 μg/mL in Tris pH 9.0) into a fluorescent indicator.

Measurements of overall titers to viral vectors

Assays of antivaccinia and anti-fowlpox IgG titers were previously reported (23, 24, 34).

Statistical analyses

Training set leads were identified as statistically significant survival differences between subjects with high versus low prevaccination antibody levels (nonparametric log rank P value less than 0.05 for Kaplan–Meier survival estimator). To avoid noise at low antibody signals, a minimum threshold (seven times the background signal) was set for stratifying subjects. In addition, all strata needed ≥7 subjects as disproportionate strata (e.g., 5 vs. 23 subjects) show chance survival differences more frequently than predicted by log-rank P values.

Because of the potential for overfitting of data, lead validation required consistent, statistically significant survival differences after accounting for multiple comparisons by permutation testing (34). Probability of type I error (α) was estimated as the frequency of random permutations (3,000 runs) with ≥1 lead showing survival differences, at thresholds determined by the training set, with equal or better significance than observed for the lead (e.g., for BG-A<sub>ant</sub> IgM, difference in median survival = 14.7 months and P = 0.005).

Correlations between antiglycan antibodies and clinical or other immunologic data were assessed by nonparametric Kendall correlations (τ) and their associated P values calculated using Partek Genomics Suite.

Results

Prevaccination antibodies to blood group A predict postvaccination survival

Serum antiglycan antibodies of PROSTVAC-VF recipients were profiled with glycan microarrays (Fig. 1) containing 171 structurally distinct glycans (Supplementary Table S1; refs. 26, 29, 30). We tested for survival correlations with antiglycan antibody levels in 2 stages (training and validation) to reduce false positives common when conducting multiple comparisons. The training stage used sera from 28 subjects enrolled in a single center, phase II study of PROSTVAC-VF for metastatic castration-resistant prostate cancer (24). Next, potential biomarkers from the initial training set were validated in an independent set of 76 patients from a separate, multicenter phase II study (23). As controls, samples were obtained from 37 prostate cancer subjects inoculated with wild-type vectors (vaccinia and fowlpox lacking the 4 transgenes) coadministered with saline injections instead of GM-CSF (23). In each case, all clinical data were blinded until after completion of antibody profiling.

Of 39 leads identified by the training set (Supplementary Table S2), only prevaccination levels of serum IgM antibodies to blood group A trisaccharide [BG-A<sub>ant</sub> = GalNAcα1-3(Fucα1-2)Galβ1] were statistically significant in both the training (P = 0.04; Fig. 2A) and validation sets (P = 0.005; Fig. 2B) after correcting for multiple comparisons (α = 0.036). That is, there was a 3.6% likelihood that chance would produce a survival correlation for any lead equivalent to or better than the survival correlation observed for anti-BG-A IgM. Median survival for subjects with abundant IgM for BG-A<sub>ant</sub> was significantly longer than for subjects with...
little or no anti-BG-Atri IgM. Upper quartiles for prevaccination anti-BG-Atri IgM survived nearly twice as long as the lowest quartile [median survival of 13.9 vs. 33.7 months in training set ($P = 0.05$); 17.1 vs. 31.0 months in validation set ($P = 0.01$)]. In addition, median actual survival for the upper quartiles was 11.5 to 15.7 months longer than their median Halabi predicted survival (training = 18.0 months; validation = 19.5 months) and 14.4 to 17.1 months longer.
than the median survival of controls (16.6 months). There
was no survival correlation with anti-BG-Atri IgG.

Our 2-stage analysis was designed to validate potential
biomarkers at the same threshold in both training and
validation sets to guard against overfitting. The small
training set, however, was not adequately powered to
evaluate extreme cutoff values as estimates based on
strata containing few subjects are prone to small sample
size effects (35). The larger number of subjects in the
validation set allowed us to more accurately evaluate
cutoff values for Kaplan–Meier analysis. At the optimal
cutoff, where approximately 22% of the patients were
below the threshold, the survival correlation was even
stronger ($P = 3\times10^{-6}$; Fig. 2C). Therefore, performance of
anti-BG-Atri IgM as a biomarker may be improved as
larger numbers of patients are evaluated and the cutoff is
further refined.

**Anti-BG-Atri IgM are prognostic specifically for
vaccinated subjects**

As a first step in investigating the biologic basis for
improved survival in subjects with abundant serum levels
of IgM for BG-Atri before vaccination, we assessed whether
this correlation was specific for vaccinated subjects or
whether anti-BG-Atri IgM are generally prognostic. We
profiled serum antiglycan antibodies of control subjects
inoculated with wild-type viral vectors. There was no
correlation with survival for subjects treated with control
vectors (Fig. 2D); therefore, levels of anti-BG-Atri IgM
were specifically predictive of survival after treatment
with PROSTVAC-VF. Also, we evaluated relationships
between anti-BG-Atri IgM levels and measures of disease
severity, such as PSA level and Gleason score. Third, we
tested for associations with prognostic factors, such as
Halabi predicted survival (36) and age. No correlation
with these factors was observed (Fig. 3), indicating the
link between survival and anti-BG-Atri IgM is unlikely to
be an epiphenomenon.

**Array’s diversity helps refine structure of antigen
associated with improved survival**

High-throughput glycoimmunomic profiling provided
considerable information about the binding properties of
the antibody populations associated with vaccine efficacy.
The blood group A determinant is defined as the trisaccharide, GalNAcα1-3(Fucα1-2)Gal; however, this trisaccharide can be found in nature attached to various oligosaccharide chains. Six distinct tetrasaccharide variants of the blood group A antigen have been described (referred to as blood groups A1 through A6). In addition, the blood group H and B antigens share considerable structural similarities with the blood group A antigen. The array contained many structurally related glycans (Fig. 4), but survival correlated with prevaccination levels of IgM to BG-Atri uniquely. Interestingly, all substitutions, eliminations, or truncations of BG-Atri weakened the survival correlation (Table 1). The low degree of correlation between antibody binding for structurally related glycans indicated that distinct antibody populations recognize each structural variant. Improved survival, in particular, was not associated with antibodies for a common blood group A variant in humans (BG-A1 = GalNAcα1-3(Fucα1-2)Gal[1-3GlcNAc][1-3Gal[1]), which are strongly associated with blood type (29) and previously have been found to be distinct from anti-BG-Atri IgM (37). Antibodies to blood groups B and H did not correlate with survival. There was no survival correlation for the terminal monosaccharide GalNAc-α or for peptides containing the tumor associated Tn antigen (GalNAc-α linked to serine or threonine). In addition, IgG antibodies for BG-Atri did not correlate with survival, although serum levels and variation between subjects were similar for IgG and IgM antibodies. Taken together, enhanced vaccine efficacy seems most closely associated with IgM antibodies for a blood group A–like glycan distinct from human BG-A1.

**Discussion**

Cancer vaccines can produce durable responses in some patients, but others receive little or no immediately observable benefit. Critical determinants of *in vivo* vaccine efficacy are poorly understood. In this study, we show that preexisting serum antibody levels to BG-Atri correlate positively with overall survival for patients treated with PROSTVAC-VF. The correlation was statistically significant in patients from 2 separate phase II clinical trials encompassing over 100 patients and remained statistically significant even after correcting for multiple comparisons. In addition, the survival correlation was specific to PROSTVAC-VF as antibody levels were: (i) independent of disease severity (PSA levels, Gleason score), (ii) independent of other prognostic factors (Halabi predicted...
survival and age), and (iii) not correlated with survival in control patients inoculated with wild-type viral vectors lacking PSA and costimulatory transgenes.

The survival correlation of anti-BG-Atri IgM in vaccinated subjects is intriguing. BG-Atri is the terminal trisaccharide portion of the blood group A antigen, which is expressed at high levels on red blood cells, epithelia, epidermis, and mucins of blood type A and AB individuals (43). Although the biologic roles of ABO blood group antigens are still not well understood, it is known that antibodies to nonself ABO blood group antigens develop early on in life and are maintained at relatively constant levels in healthy adults (29, 44). These antibodies have been proposed to provide a barrier to curb the spread of certain diseases across individuals of different blood types (45). Moreover, strong immune responses to “foreign” ABO blood group antigens limit incompatible blood transfusions and organ transplants.

In the case of PROSTVAC-VF, the anti-BG-Atri IgM bind a blood group A-like glycan on the surface of the poxvirus. The glycan is presumably carried over from chicken embryo dermal cells, the host cells used for production of PROSTVAC-VF. Antibody bound to viral vectors can influence immune responses in a variety of ways. For example, emerging evidence indicates that certain types of preexisting antibodies, referred to as “natural antibodies” or B-1 class antibodies, may augment vaccine responses by linking innate and adaptive immunity (46). These natural antibodies, which include many antiglycan antibodies, have been shown in murine models to enhance immune responses in a complement-dependent manner (47). Studies on the α-gal antigen (48, 49) provide additional precedent for carbohydrates as vaccine adjuvants. Binding of preexisting antibodies to the α-gal glycan on vaccines has been shown to enhance antigen uptake and presentation and induce stronger immune responses (50, 51). In fact, attachment of the α-gal glycan to antigens can significantly enhance their immunogenicity (50, 51). Anti-BG-Atri IgM binding to PROSTVAC-VF’s viral vectors may increase immunogenicity in a similar manner.

Although other preexisting serum antibodies may bind the poxvirus vectors, anti-BG-Atri IgM seem to be unique. Preexisting antibodies for a vaccine’s viral vectors have long been investigated as potentially influencing vaccine efficacy. For example, several studies have evaluated relationships between overall antibody titers to poxvirus vectors and survival (23, 24). These studies have primarily grown out of concerns that preexisting neutralizing antibodies to the vectors would render virus-based vaccines ineffective; however, these studies showed no correlation between overall titers to poxvirus vectors and survival.

| Table 1. Structural specificity of antigen associated with improved overall survival |
|----------------------------------|------------------|------------------|-----------------|
|                                | Minimum P value  | Stratifications | Correlation (r) |
|                                |                  | with P < 0.005   | with BG-Atri     |
| BG-Atri (4/BSA)                | 1.39E-07         | 26               | 1               |
| GaINAcα1-6Galβi (22/BSA)       | 1.96E-05         | 5                | 0.59            |
| GaINAc-α (4/BSA)               | 5.02E-04         | 2                | 0.29            |
| BG-Atri (17/BSA)               | 0.0015           | 0                | 0.29            |
| Ac-A-Tn(Thr)-S-G (5/BSA)       | 0.0017           | 0                | 0.58            |
| GaINAc-α (22/BSA)              | 0.0019           | 0                | 0.26            |
| GaINAcα1-6Galβi (4/BSA)        | 0.0061           | 0                | 0.26            |
| Ac-A-Tn(Thr)-S-G (23/BSA)      | 0.0067           | 0                | 0.34            |
| Ac-A-Tn(Thr)-S-G (8/BSA)       | 0.0073           | 0                | 0.26            |
| BG-A1                           | 0.0083           | 0                | 0.05            |
| BG-H2                           | 0.014            | 0                | 0.26            |
| Fsα (31/BSA)                   | 0.019            | 0                | 0.43            |
| BG-Btri                         | 0.029            | 0                | 0.35            |
| Fsα (21/BSA)                   | 0.048            | 0                | 0.43            |
| BG-A + Lewis B hexa             | 0.063            | 0                | 0.62            |
| Fsα (4/BSA)                    | 0.066            | 0                | 0.43            |
| BG-H1                           | 0.094            | 0                | −0.07           |
| BG-Btri                         | 0.15             | 0                | 0.29            |

NOTE: For each glycan structurally similar to BG-Atri, the table contains the minimum log-rank P value of all possible Kaplan–Meier survival curves stratified by IgM antibody levels for that particular glycan. Also listed are the numbers of stratifications (65 possible containing 6–70 subjects) whose Kaplan–Meier survival curves have log-rank P values < 0.005. The degree of cross-reactivity of anti-BG-Atri IgM for structurally similar glycans was estimated by Kendall correlations (r) between IgM antibodies levels for BG-Atri and related glycans.
Moreover, there are numerous other glycans on the pox-virus surface. Of the antiglycan antibody populations detectable on our array, however, only preexisting anti-BG-Atri IgM correlated with clinical outcome, possibly due to a higher level of expression of the corresponding antigen on the viral surface than other glycans, a unique location of the antigen, or more substantial variability in anti-BG-Atri IgM levels among patients. It is important to note that antibody-mediated immune enhancement could occur in patients receiving either PROSTVAC-VF or the wild-type control poxviruses; however, the correlation of anti-BG-Atri IgM with survival was only observed in patients treated with PROSTVAC-VF. Therefore, these antibodies correlate with clinical outcome only when coupled with immune stimulation toward a tumor antigen.

Our results have a number of important implications. They provide the first evidence that anticarbohydrate antibodies could be useful biomarkers for PROSTVAC-VF. In principle, potential patients may be prescreened for IgM antibodies to BG-Atri as one criterion for treatment with PROSTVAC-VF. Although our study comprised over 100 vaccinated patients, as well as controls to show the prognostic specificity of anti-BG-Atri IgM, additional prospective studies with larger numbers of patients and control subjects will be an important focus of future research. The ongoing phase III clinical trial on PROSTVAC-VF is anticipated to enroll 1,200 patients, and samples from this study could provide additional validation of anti-BG-Atri IgM as a predictor of clinical response (25).

Second, glycan composition and consistency may be critical features of vaccine potency. Factors that influence glycan expression, such as type of host cell and growth conditions, may significantly affect clinical outcomes for poxvirus-based vaccines and optimizing these factors may improve efficacy. Analysis of a viral vector’s glycans also may be an important quality control assessment. Third, anti-BG-Atri IgM may be relevant to other pox-based therapies, such as other cancer vaccines [e.g., PANVAC (52) and rV-NY-ESO-1 (53)], oncolytic poxviruses (54, 55), and HIV vaccines [e.g., ALVAC-HIV (56)]. Because these poxviruses are often produced in chicken-derived cells, they also could display blood group A–like glycans.
on their surfaces. Therefore, antibodies to BG-Atri could influence their clinical efficacy too. Analogously, antibodies levels to blood group B may be relevant for vaccines propagated in other cell lines that express blood group B. By addressing technical challenges in studying carbohydrate–protein interactions, glycan microarrays provide a powerful tool for exploring connections between antiglycan antibodies and disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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