Immunosignatures can predict vaccine efficacy

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The development of new vaccines would be greatly facilitated by having effective methods to predict vaccine performance. Such methods could also be helpful in monitoring individual vaccine responses to existing vaccines. We have developed "immunosignaturing" as a simple, comprehensive, chip-based method to display the antibody diversity in an individual on peptide arrays. Here we examined whether this technology could be used to develop correlates for predicting vaccine effectiveness. By using a mouse influenza infection, we show that the immunosignaturing of a natural infection can be used to discriminate a protective from nonprotective vaccine. Further, we demonstrate that an immunosignature can determine which mice receiving the same vaccine will survive. Finally, we show that the peptides comprising the correlate signatures of protection can be used to identify possible epitopes in the influenza virus proteome that are correlates of protection.

peptide microarray | systems vaccinology | epitope prediction | antibody repertoire | immune profile

Vaccine efficacy trials rely upon direct measures of candidate-elicited protection, namely survival from challenge in laboratory settings or reduced incidence of disease in clinical trials, to determine whether a vaccine candidate is useful. In diseases such as malaria, human challenge is permitted and can be conducted at a defined time point following immunization (1). For some infectious diseases such as HIV, live human challenge is unethical, so protection is determined through reduced incidence of disease in a susceptible population (2). Biothreats and emerging agents pose unique challenges; they are dangerous, lack animal models, and do not have endemic populations that can be epidemiologically studied. These considerations highlight the need to develop a platform for the evaluation of vaccines in natural hosts without pathogen challenge. Efforts to develop such a platform have focused on identifying correlates of protection. Correlates of protection are immune responses that are specific products of the vaccine and statistically associated with protection (3–5). Threshold measurements above which an individual will be protected from disease are determined for each correlate and used to evaluate the response to candidate vaccines (3). Assays designed to measure correlates of protection are disease-specific and cannot be used to evaluate vaccines to other pathogens. An ideal platform would be highly accurate, inexpensive, and broadly applicable across disease categories. We have developed the "immunosignaturing" diagnostic technique to display the circulating antibody diversity present in sera while meeting these criteria (reviewed in ref. 6). Here we evaluate whether immunosignatures can characterize and stratify vaccines into those that are efficacious and those that are not.

Vaccine efficacy against an infectious disease can often be distilled to the presence of a particular antibody species in the vaccine-generated immune response (4). In infectious disease, these antibodies are neutralizing antibodies that have been raised against pathogenically important epitopes of a pathogen. For known pathogen epitopes, it is usually straightforward to develop an ELISA-based test to detect these antibodies. For diseases for which single correlates of protection have failed to be found, multiplexed assays have been tried in attempts to identify indicative sets based on many antigens. Protein microarrays have enabled higher-throughput screening, but obtaining properly folded candidate proteins remains challenging, thereby limiting the detection of conformational epitopes. Further limiting the utility of protein microarrays is the absence of carbohydrate antigens, posttranslational modifications, and unannotated proteins. Tiled peptide arrays have numerous advantages over whole protein: peptides can be synthesized in large scale; they can contain overlapping sequences, ensuring adequate coverage of the annotated portions of the proteome; and they are quite stable, with no need for a tertiary conformation. However, structural epitopes are unrepresented, and a new array must be made for every pathogen tested, limiting the breadth of application.

Immunosignaturing enables the broad information content of the circulating antibody repertoire to be examined at one time. This approach capitalizes on the ability of antibodies to bind multiple targets including both the original epitope and mimetic peptides (7). Antibodies are captured on a large number of long, pseudorandom, nonnatural sequence peptides, which yield mimetics covering linear and conformational epitopes as well as possessing the ability to detect antibodies against sugars, phosphates, and other posttranslational modifications. The circulating antibody repertoire is spread across the array based on antibody binding preference, producing a true signature of an individual's reactivity profile, including any disease or vaccine they have had. Despite the individual influences on the total immunosignature, a subset of peptides stands out and is informative for a given disease. Currently, the Center for Innovations in Medicine (CIM) CIM10K, a microarray of ten thousand 20-aa peptides, is used for the diagnosis of human disease. Thus, far, immunosignatures have demonstrated a high degree of sensitivity and specificity for classifying human, dog, and mouse infections (8) and chronic (9) and genetic diseases (10, 11). On a finer scale, individual monoclonal antibodies bind to a surprisingly large number of

Significance

Vaccines have been the most important medical intervention developed, yet vaccines for many diseases are still needed. Despite its success, the process to develop a vaccine remains empirical, resting on measuring the number of vaccinees that incur or do not incur an infection. Here we test in the mouse flu model whether the "immunosignature" diagnostic technology could be applied to predict vaccine efficacy. Immunosignatures are produced by profiling the antibody repertoire of an individual on a chip arrayed with nonnatural sequence peptides. It is attractive in that it is a simple but comprehensive measure of the complexity of the humoral response. We found that immunosignatures are a promising approach to predicting whether a vaccine will confer protection.

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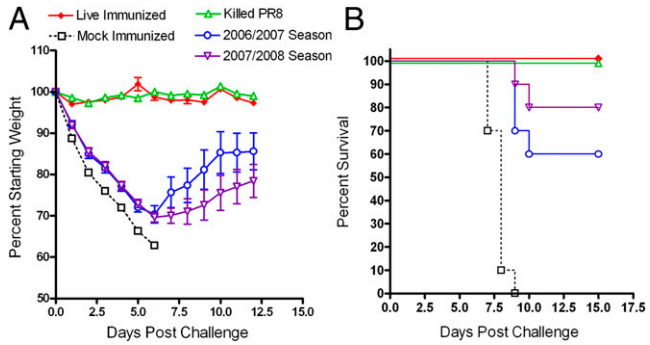

Fig. 1. Outcomes of immunized mice following lethal challenge with influenza A/PR/8/34. Mice were immunized with PBS solution (mock), a live sublethal dose of A/PR/8/34, inactivated A/PR/8/34, the 2006/2007 seasonal influenza vaccine, or the 2008/2007 seasonal influenza vaccine. Mice were challenged intranasally with 5×10^5 pfu per mouse. The average daily percent starting weight is graphed in *A*, where the average is calculated based on the surviving mice and error bars represent the SD. Survival curves are presented in *B* and represent the percentage of mice surviving following challenge.

random-sequence peptides and are detectable within a complex mixture of competing antibodies (7, 12). On a grosser scale, immunosignatures can resolve a polyclonal response to individual peptide epitopes (12, 13). In the preliminary demonstration of immunosignatures, human seasonal influenza vaccinees were separated based on their pre and post vaccination immunosignatures (8). This observation and the ability to detect antibodies to specific epitopes against complex mixtures lead to the hypothesis that immunosignatures can be used to predict vaccine efficacy.

Here we address the hypothesis that the immunosignaturing platform can quantitatively distinguish protective from nonprotective vaccines. We chose a murine influenza model, A/PR/8/34, to test the concept. This model has several significant advantages in terms of vaccine studies. First, the virus is lethal in unprotected mice; killed virus and sublethal infection typically lead to complete protection, and there are a number of influenza strains which convey partial protection (14). In addition, there is a detectable antibody titer in infected and vaccinated animals. Within this model, the immunosignatures to effective vaccines and partially protected vaccines were examined. By using this model, we asked whether the immunosignature to a natural infection could predict vaccine effectiveness. We also determined if the variable levels of protection seen in individuals receiving the same vaccine could be predicted. Finally, we show that peptides identified as part of the immunosignature can be used informatically to identify the relevant viral antigens.

Results

Study Plan. To test the ability of the immunosignature to predict vaccine efficacy, a flu model system was used. If differences in immunosignatures correspond to protective epitopes, the immunosignature should predict which vaccines are protective. Five groups of 10 female BALB/c mice were immunized, individually bled, and challenged with the influenza H1N1 A/PR/8/34 (PR8) virus. Three groups were also vaccinated with inactivated viruses. These were formalin-inactivated PR8 (killed PR8) and the commercially available 2006/2007 and 2007/2008 seasonal trivalent influenza vaccines. The two seasonal influenza vaccines share the same A/Wisconsin/67/2005 (H3N2) and B/Malaysia/2506/2004 but vary in the H1N1 portion, containing A/New Caledonia/20/99 and A/Solomon Islands/3/2006, respectively. In addition to a mock vaccination group, the fifth group was vaccinated a single time with a sublethal dose of the live PR8 virus.

The mice were challenged with two to five mean lethal doses of active PR8, and results are presented in Fig. 1. No mice immunized with killed PR8 or given a sublethal infection died of the challenge, and none of these mice had a symptomatic infection as evidenced by the absence of fur ruffling and weight loss. The two seasonal vaccines were partially protective, resulting in 60% and 80% survival. All surviving mice had significant weight loss. Serum was collected 2 d before challenge and used to probe the CIM10K array and establish the immunosignature of each mouse. The challenge responses represented in Fig. 1 are the basis for testing the value of immunosignatures to answer the following questions: Can the natural infection signature be used to distinguish the difference in efficacy between the sublethal/killed PR8 vaccines and the seasonal vaccines? Even though the sublethal challenge and the killed PR8 vaccines produce full protection, are their immunosignatures distinguishable? Finally, can the immunosignatures be used to separate the mice that do survive and those that do not in the seasonal flu vaccine groups?

Baseline Comparison with Standard Immunological Assays. To create a baseline for comparison with immunosignatures, serum antibodies against the viruses were assessed by ELISA 2 d before challenge. As evident in Fig. 2, only the mice receiving the live vaccine or the killed PR8 vaccine had detectable antibodies against PR8. The two seasonal vaccines, which were only partially protective against the PR8 challenge, did not have a detectable response to PR8.

Live and Inactive Influenza Immunizations Produce Different Immunosignatures. The live and killed PR8 vaccines were equally protective against challenge. The ELISA against whole virus in Fig. 2 demonstrated that the live and inactivated influenza immunizations produce different intensities of antibody response. We wished to determine if the immunosignatures were also different between these two groups. The differences in peptides recognized by each vaccine group vs. naive group are seen in a scatterplot in Fig. 3*A*. By using selection criteria of false discovery rate-corrected $P < 0.05$ and fold change >1.3 fold, serum from live influenza recognizes 10.75 times the number of peptides as the inactive vaccine serum. The two vaccines have seven peptides recognized in common, one would expect less than one peptide recognized by chance between similarly sized lists (Fig. 3*B*). A principal components analysis (PCA) plot displays the relative difference among and between groups using variance as the *x* and *y* scalar values. All 593 peptides recognized by either group of mice clearly separate the live from inactive immunized animals (Fig. 3*C*). A support vector machine (SVM) shows 0% leave-one-out cross-validation (LOOCV) error when asked to predict the classes. Analysis of the overlapping peptides shows the live- and inactive-vaccinated mice cluster together, and are separate from the mock-immunized mice (Fig. 3*D*). The larger number of peptides in the live vaccine immunosignature may result from the dose-amplifying effect of viral replication or additional epitopes not present in the inactivated, and presumably disassembled, virion. This difference between live and inactive PR8 immunosignatures is consistent with different gene expression profiles between live and inactive influenza vaccines (15). Taken together, these

Fig. 2. Whole virus-specific IgG measured in an ELISA. Before challenge, serum was collected from all mice. The amount of antigen-specific circulating IgG was measured for inactive PR8 and the 2006/2007 and 2007/2008 seasonal vaccines by endpoint titer and is graphed. Error bars are the SD of triplicate measurements of pooled sera.

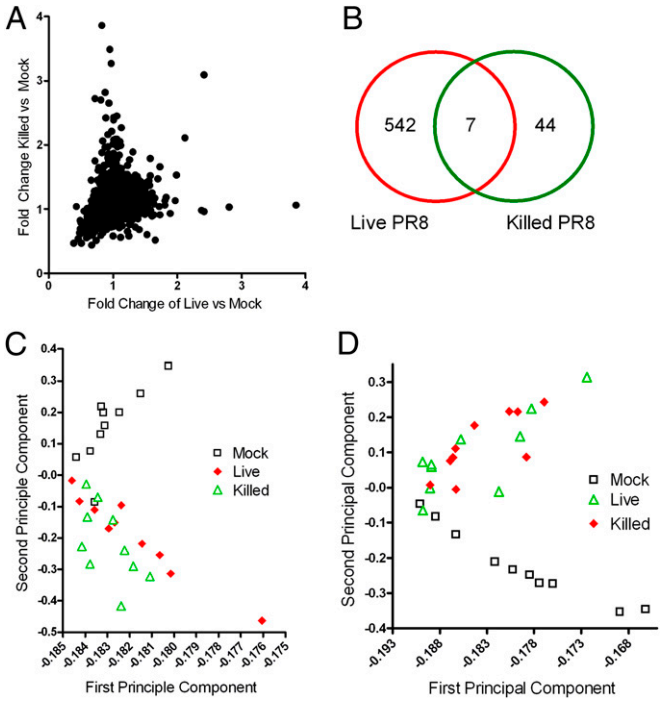

Fig. 3. Comparison of the immunosignature to live and inactive Influenza A/PR/8/34. The first analysis of the CIM10Kv3 array data were to compare the immune response to the live and inactive PR8 vaccines. A scatterplot of the fold change ratio of each vaccine to naive mice is shown in *A*, where the live PR8 is on the *x* axis and the inactive PR8 is on the *y* axis. The overlap between peptides that are significantly different ($P < 0.05$ with Benjamini and Hochberg Multiple Test Correction) above 1.3 fold in each vaccine are presented in the Venn diagram in *B*. The variance in the immune responses between individuals is shown in the PCA analysis in *C* for all peptides and (*D*) for the overlap, where the first two principal components are plotted and individuals colored by vaccine.

data demonstrate that the two protective vaccines have quite distinct immunosignatures.

The Immunosignature Can Distinguish Closely Related Vaccines. To evaluate the capacity of immunosignaturing for fine-scale profiling, we probed the CIM10K arrays with serum from mice immunized with the inactivated influenza vaccines. Two versions of the CIM10K array were used: pooled samples were tested on the CIM10K version 1 (CIM10Kv1) and individual samples were tested on the CIM10K version 3 (CIM10Kv3). As the CIM10Kv3 is our current array and incorporates numerous technical improvements, it was chosen to evaluate the individual mice. The first comparison asked for peptides different from the grand mean across the three vaccines by using one-way ANOVA at $P < 0.0005$. This comparison yielded 55 peptides capable of separating the three vaccines with 0% LOOCV error in an SVM (Fig. 4*A*). The second comparison compared each vaccine separately against the mock-immunized mice by using the Student *t* test. The number of significant peptides compared with mock were different between vaccines. Overlap between the two sets of peptides is shown in the Venn diagram in Fig. 4*B*. Notably, the overlap is greater than expected by chance, and even more overlap is seen between the two seasonal vaccines than with the killed PR8. The two seasonal vaccine formulations differ in the H1N1 strain included. This pattern is consistent on the CIM10Kv1 and CIM10Kv3 microarrays. This demonstrates that immunosignatures are sensitive enough to detect subtle differences in vaccine compositions.

The Immunosignature of a Known Protective Response Can Predict Outcome Following Challenge. Having demonstrated that the immunosignature can detect subtle differences in the antibody response to closely related vaccines, we sought to test whether the immunosignature of a known protective response could predict vaccine efficacy. The SAM algorithm uses a permutated *t* test and was used to select 25 peptides capable of distinguishing live from mock immunized as the training set with a false-positive rate of 1 peptide in 25 (4%). These 25 peptides included the overlap peptides between the live and killed PR8 immunosignatures. To overcome the influences of varying affinities for peptides, we used a binary classifier that bins array features based on whether a certain cutoff score has been reached. These binary scores were used to calculate the group average of pairwise Hamming distances as the number of binary differences between immunosignatures shown in Table 1. Seasonal vaccines were used as the test set on the same 25 peptides. The mice immunized with killed PR8 were found to be closer to the live immunized mice and further from the mock-immunized than those receiving the seasonal vaccines. This fits with the inactive PR8 imparting complete symptom-free protection, whereas the seasonal vaccines only afforded partial protection. Immunosignature-based prediction of the killed PR8 as the most protective vaccine reflects the relative ELISA titers. Had the immunosignature been the only assay used, it would have picked the correct vaccine. The data demonstrate the ability of the immunosignature to aid in vaccine development by selecting the vaccine with the highest protective efficacy.

Seasonal Vaccine Recipients Have Distinct Immunosignatures, Which Correlate with Outcome Following PR8 Challenge. Mice immunized with the seasonal vaccines were partially protected against challenge with the PR8 strain. We posed whether the immunosignatures of surviving mice were distinctive. We first evaluated the sera in an endpoint ELISA against whole PR8 virus (Fig. 5*A*) and did not observe an explanatory trend within the groups. The immunosignatures of all surviving mice from both seasonal vaccines were subsequently compared with all the seasonal vaccine recipients which succumbed. By using a two-tailed *t* test with a cutoff of $P < 0.005$, 94 peptides were identified as significantly different and were capable of a 100% LOOCV accuracy using SVM. In a PCA plot, the surviving mice grouped with the mice immunized with killed PR8 (Fig. 5*B*). Notably, the PR8-immunized mice were not included in the selection of these 94 peptides. This suggests that

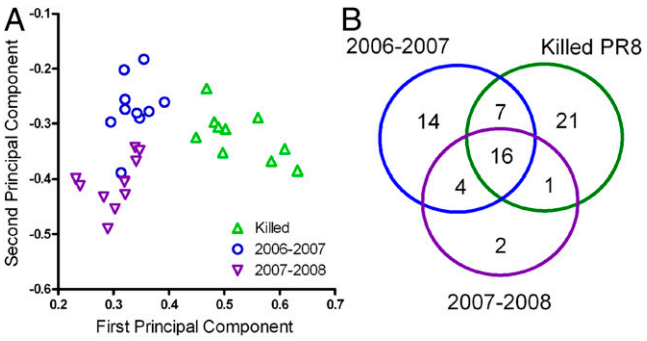

Fig. 4. The immunosignature can distinguish weaker inactive vaccines from a more potent one. The immunosignature on the CIM10Kv3 was compared between the two seasonal vaccines and the killed PR8 vaccine first in an ANOVA in which 55 peptides at a $P < 0.0005$ (five false positives) were capable of separating the three vaccines. Variance among individuals is represented in a plot of the first and second principal components in *A*. Comparisons between vaccinated and naive mice were made by using pooled sera on the CIM10Kv1 using a minimum 1.3-fold increase in normalized fluorescence units in sera from immunized over mock and a *P* value of less than 0.05 using the Benjamini and Hochberg multiple test correction. Overlap between these lists is shown in the Venn diagram in *B*.

Table 1. Average pairwise vaccine immunosignature Hamming distance

Group	Distance From Mock	Distance From Live PR8
Mock	3.4 ± 1.2	21.0 ± 3.0
Live	21.0 ± 3.2	4.5 ± 4.4
Killed PR8	8.3 ± 4.0*	16.4 ± 4.1†
2006/2007	5.9 ± 4.7	18.7 ± 5.0
2007/2008	5.6 ± 1.6	19.0 ± 3.1

Average Hamming distances ± SD are shown. The log2 of ratios between individuals were calculated for each of the peptides capable of distinguishing live from mock-immunized mice by using Statistical Analysis of Microarrays (SAM). Ratios were binned as 1 or 0 based on a cutoff of the peptides 10th percentile in the live-immunized mice. The Hamming distance was calculated by using the binary scores.

*Statistically distinct from the seasonal and mock vaccines by two-tailed *t* test: $P = 1.39 \times 10^{-6}$.

†Statistically distinct from the seasonal and mock vaccines by two-tailed *t* test: $P = 6.5 \times 10^{-5}$.

immunosignatures could be used to predict the individual outcome for vaccinees upon infection.

The Immunosignature Can Be Bioinformatically Tracked Back to the Epitopes on the A/PR/8/34 Proteins. The experiments described earlier indicate that immunosignatures can measure correlates of protection. It would be useful if these correlates could predict the protective epitopes in the virus. We previously demonstrated that the immunosignature of PR8 was comprised of peptides whose cognate antibody could be absorbed out by inactive virus (8). Recently, we have developed the bioinformatic tool called GuiTope, which predicts the epitope bound by antibodies by using a list of peptides selected from a library, including peptide arrays (16). To assess the breadth of epitopes recognized by the inactive vaccines, we reduced our selection of peptides to those that had increased signals following immunization. Peptides binding antibodies raised by immunization with killed PR8 were searched against the PR8 protein sequences and the results for HA and neuraminidase (NA) plotted in Fig. S1. Because of the close homology between influenza strains, common epitopes were predicted, yet the killed PR8 vaccinated mice recognized unique sequences. To test these predictions, the pooled sera of the immunized mice were used to probe a tiled peptide array containing most of the PR8 HA and NA sequences. From the sequences present on the tiled peptide array, all the predictions were supported but not all epitopes were predicted. Exclusion of these epitopes from the predicted list may be caused by the stringent false-discovery corrections used to select the peptides. If the false-discovery correction is removed, the percentage of epitopes in agreement between the GuiTope prediction and the epitope array increases. Interestingly, only the mice immunized with killed PR8 were predicted to bind epitopes on NA, including 203-SWRKKILRTQES-209, whose homolog in the 2009 A H1N1 virus is a known neutralizing epitope (17). Not all peptides included in the immunosignature were predicted by GuiTope to align to actual sequences. These peptides may be recognized by antibodies to conformational epitopes. Nonetheless, these results demonstrate that immunosignatures can accurately detect antibodies raised elicited endogenously against linear epitopes of biological immunogens.

To extend the analysis of potential correlates of protection, an informatics analysis of the 94 peptides distinguishing the surviving and nonsurviving mice from each other was conducted. Thirty-eight of these peptides that were increasingly recognized in the survivors vs. those that died. These peptides were used to GuiTope search the PR8 (HA and NA) protein sequences. A search of the NA sequence identified a strong alignment of seven peptides to residues 195 to 219 (Fig. 5*C*), which spans a known neutralizing epitope (17). This neutralizing epitope was recognized by the surviving mice, including those immunized with killed PR8, but not the naive mice or those that died of challenge (Fig. 5*D*). Antibodies against NA are known to provide protection in the absence of HA antibodies (18). Taken together, these examples demonstrate that immunosignatures have the resolving power to discern differences in the antibody response that correlate with vaccine efficacy.

Discussion

In this report, we tested in principle the ability of the immunosignature platform to inform vaccine development. First, we showed that sera from mice immunized with trivalent flu vaccines containing two identical and one highly related viral strain can be distinguished on the CIM10K array. Second, we demonstrated that the signature of a protective response generated by a live infection can predict the efficacy of a vaccine with unknown characteristics, even when it is a formalin-fixed formulation. Third, we demonstrated that an immunosignature can identify specific differences in individual mouse reactivities to the same vaccine that correlate with its protection following viral challenge. Finally, we demonstrated that the informative peptides comprising the immunosignature have similarity to pathogen epitopes that may be the protective antigens. These results indicate that the breadth of information displayed by an immunosignature may significantly reduce the costs and timeframes committed to vaccine testing.

Application of immunosignature diagnostics to evaluating vaccines adds a unique dimension to systems vaccinology. Systems vaccinology takes a holistic systems biology approach to identify correlates of protection (15, 19, 20). By broadly measuring

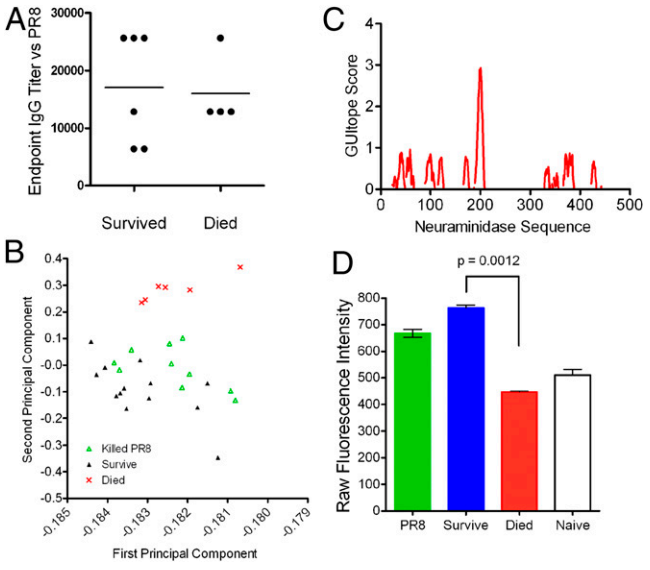

Fig. 5. The immunosignature predicts that antibody cross-reactivity to NA195-219 was important in protecting seasonal vaccine recipients from the PR8. The whole-virus ELISA endpoint titers for the 2006/2007 vaccine recipients that survived or died following PR8 challenge are shown in *A*, where the horizontal line represents the group mean and each point represents an individual mouse. The immunosignature was compared between the seasonal vaccine recipients for both years that survived or died of challenge. A Student *t* test with a *P* value of 0.005 identified 94 peptides that were significantly different between the two groups. The variance among all individuals receiving an inactive vaccine is presented in *B* as a plot of the first and second principal components. The 38 peptides at least 1.3 fold less recognized by those that died were used to predict the epitope in NA (*C*), where the GuiTope score is on the *y* axis and amino acid on the *x* axis. Antibody reactivity from pooled sera to the strongest predicted epitope are plotted in *D* as the mean ± SD of replicate arrays.

IMMUNOLOGY

cytokine response, changes in host gene expression, cell population changes, and ELISA data, the goal is to identify a signature that predicts whether a protective immune response is generated. Systems vaccinology was able to distill the multifaceted immune response to the yellow fever YF-17D vaccine to a signature capable of predicting the production of neutralizing antibodies and cytotoxic T cells (19). In contrast, we used only one platform for the evaluations of the vaccines. In a study of seasonal influenza vaccines, the gene expression profile elicited by one seasonal vaccine was able to predict with greater than 90% accuracy which individuals in the following year would generate an antibody titer above the seroconversion threshold (15). However, gene expression profiles indirectly measure the antibody response, not the specificity of antibody reactivity. In the case of attenuated dengue fever vaccines, gene expression profiles were identical for different serotypes (21) and are not descriptive of the adverse response of vaccination with the wrong serotypes of virus (22, 23). Immunosignatures should provide a valuable addition to systems vaccinology, and at times may alone be sufficient for evaluation.

Proteome arrays, which focus on a narrow set of the pathogen proteome, have been used mainly as an antigen discovery tool for inclusion in a candidate vaccine (24–30), with limited use in comparing vaccines (31). Not all antibodies detected against a single protein or epitope are equal in binding strength, specificity, or functional capability. These measures report the total of epitope-specific neutralizing antibodies, epitope-specific non-neutralizing antibodies, plus those serum antibodies that are cross-reactive yet raised by unrelated immunogens. Discernment of these antibody populations on protein and tiled peptide microarrays is easier but not complete. These arrays only represent the sequences included in the array design and do not represent conformational epitopes, nonprotein antigens, and unannotated proteins, which may misidentify a known protein on microarrays (13). Secondary infections are a frequent cause of mortality in HIV and synergistically increase lethality in influenza. A recent report demonstrated that vaccination with the influenza vaccine, but not a pneumococcal vaccine, reduced pneumococcal carriage following influenza infection (32). The portion of the vaccine response responsible for reduced carriage would not be discernible on a dedicated influenza assay while the immunosignature is capable of simultaneously detecting the combined immunosignature of multiple immunogens (7).

Detection of conformational epitopes are key to evaluating vaccines. Neutralizing antibodies against pathogens such as HIV are predominantly directed against conformational epitopes, and, in *Plasmodium chabaudi* malaria, antibody titers to linear epitopes were associated with poor vaccine efficacy whereas the antibodies raised against properly folded proteins were associated with protection (33). Given the difficulties in producing properly folded protein, peptide mimetics are an ideal means to define antibodies to conformational epitopes. Each B cell produces a single antibody species whose complementarity determining region comprises the unique set of paratopes dictated by the variable region genes. Usually only one of these paratopes is in direct contact with the immunogen; however, the other paratopes contribute to the conformation of the contacting paratope enabling proper binding. Each of these paratopes is capable of binding a distinct range of preferred mimetic peptides. The contacting paratope binds a range of mimetic peptides, which includes the neutralizing epitope and related sequences. In total, these sets of paratope-specific mimetic peptides form a descriptive immunosignature for each antibody species and is captured on immunosignature arrays without reliance on the pathogen sequence (7, 12).

In our analysis of the partially protective trivalent seasonal vaccines, the ELISA titers were unable to explain survival, and, on the tiled peptide microarrays, identified only one epitope on NA as significantly different between survival and mortality. By considering the full reactivity profile of the vaccine generated antibodies on the CIM10K arrays, the immunosignature was able to discern that the survivors had an antibody reactivity in common that was absent in those that died. Part of this immunosignature was bioinformatically tracked back to the NA epitope, whereas the remainder may result from the differently efficacious antibodies to the epitopes they held in common. This demonstrates that the immunosignature serves as a highly sensitive correlate of protection and be used to identify the responsible antigens.

Apart from vaccine development, the ability to predict whether an individual would survive a lethal infection may have application in the public health response to pandemic disease. During the onset of the swine-origin H1N1 pandemic, it was evident that a new vaccine was needed and that the egg-based methodology was less than optimal (34). A recent serological study of 1,000 donors across lifespan estimated that 25% of the population would be protected against a novel swine-origin A(H3N2) strain whereas the remainder would require additional vaccination (35). For example, in the setting of a novel pandemic strain, the prereadied immunosignature assay, which requires no stocks of the new emerging virus for analysis, could be used immediately to establish which segment of the population most needs limited vaccine resources. Selection of the most protective vaccine could be made by comparing the serum immunosignature of survivors to the previously known immunosignatures of stockpiled vaccines. Such population-wide applications would require a platform that was inexpensive and could be mass produced. Immunosignaturing could be suited for this type of use.

This report expands the range of applications for the immunosignature platform beyond diagnostics to include vaccine evaluation and selection. In a murine model of influenza, the immunosignature served as a correlate of protection and facilitated the identification of the involved epitopes. Under the accelerated-approval regulation, the Food and Drug Administration allows surrogate endpoints to be used in clinical vaccine trials (36). As an inexpensive assay, the immunosignature can be applied to hundreds and thousands of volunteers in clinical efficacy trials and serve as a surrogate endpoint. For diseases such as HIV, in which human challenge is unethical, and norovirus, in which there is no animal model, the use of immunosignatures would serve as a surrogate endpoint and a discovery tool to rapidly distill out the true correlates of protection and develop a more effective subunit vaccine. Originally designed as a diagnostic tool, the immunosignature has the additional power to simultaneously identify infections such as herpes simplex virus that confound HIV vaccine efficacy trials (37). This potentiates the preselection of a trial population without confounding factors strengthening study conclusions. Immunosignatures can extend the multivariate analysis used by systems vaccinology to all steps of the vaccine development and validation process.

Materials and Methods

Five groups of 10 female BALB/c mice were immunized and individually bled, and then were challenged with the H1N1 A/PR/8/34 (PR8) virus. Mice immunized with the killed virus preparations received 1.0 μg of seasonal vaccine or killed PR8 to be equivalent in the total amount of protein delivered. The mice received three immunizations of the killed viruses. Serum from immunized mice was used in an immunosignature assay by probing the CIM10K random peptide array. Bound IgG was detected by using a fluorescently labeled anti-mouse IgG. The resulting fluorescence intensities were analyzed to determine the unique patterns of antibody binding or immunosignature representative of each group. Lists of significant peptides were analyzed by using the GuiTope program for alignment to known PR8 epitopes. Detailed materials and methods are provided in *SI Materials and Methods*.

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