Identification and characterization of CD4+ T cell epitopes after Shingrix vaccination.

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Running Head**:** Human T cell epitope identification in Shingrix vaccinees

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Abstract

Infections with varicella zoster virus (VZV), a member of the Herpesviridae family, are associated with a range of clinical manifestations. Primary infection with VZV causes chicken pox, and due to the virus's capacity to remain latent in neurons, it can reactivate later in life causing herpes zoster (HZ), also known as shingles. Two different licensed vaccines are available to prevent HZ, either based on a live attenuated VZV strain (Zostavax) or on adjuvanted gE recombinant protein (Shingrix). While Zostavax efficacy wanes with age, Shingrix protection retains its efficacy in elderly subjects (80 years of age and beyond). In this context, studies to evaluate the potential role of T cell immunity as a correlate of protection and Shingrix efficacy are of interest. In this study, we characterized Shingrix specific ex vivo CD4 T cell responses in the context of natural exposure and HZ vaccination using pools of predicted epitopes. We show that T cell reactivity following natural infection and Zostavax vaccination dominantly targets non-structural proteins, while Shingrix vaccination redirects dominant reactivity to target gE. We mapped the gE-specific responses following Shingrix vaccination to 89 different gE epitopes, 34 of which accounted for 80% of the response. Using antigen presentation assays and single HLA molecule transfected lines, we experimentally determined HLA restrictions for 94 different donor/peptide combinations. Finally, we used our results as a training set to assess strategies to predict restrictions based on measured or predicted HLA binding and the corresponding HLA types of responding subjects.

Importance

Understanding the T cell profile associated with the protection observed in elderly vaccines following Shingrix vaccination is relevant to the general definition of correlates of vaccine efficacy. Our study enables these future studies by clarifying patterns of immunodominance associated with Shingrix vaccination, as opposed to natural infection or Zostavax vaccination. Identification of epitopes recognized by Shingrix-induced CD4 T cells vaccinees, and their associated HLA restrictions, enables the generation of tetrameric staining reagents, and more broadly the capability to characterize specificity, magnitude and phenotype of VZV specific T cells.

Introduction

VZV, or HHV3, is a dsDNA virus of about 125kb and belongs to the Herpesviridae family. The corresponding proteome encompasses 69 proteins, of which 37 are structural proteins including Envelope, Tegument, and Capsid, and 32 are non-structural proteins involved in viral replication and innate host immunity evasion (5). Three protein layers comprise a VZV particle: a nucleocapsid containing the highly conserved dsDNA genome, a tegument layer, and an envelope lipid bilayer containing viral glycoproteins (10).

Primary infection generally occurs in children prior to adolescence and clinically manifests as Varicella or chickenpox, a mild- to moderate disease with symptoms appearing 10-21 days after exposure (3). Symptoms include headache, loss of appetite, tiredness, fever, and pruritic rashes appearing on the head, torso, and extremities (3). The host immune system controls, but does not eliminate, the virus, as VZV enters a life-long latency state in the sensory neurons of the trigeminal and dorsal root ganglia (10). Usually later in life, the second disease state, Herpes Zoster (HZ) can manifest as a reactivation of latent virus that spreads throughout many neurons and along entire ganglia (10, 32, 52). Post-herpetic neuralgia (PHN) is a very common neurological complication of HZ and can persist for years. Immunocompromised patients are at greater risk for PHN, and nearly half of patients over 60 years who experience HZ will also experience PHN (47). Furthermore, half of adults who live to age 85 will experience Herpes Zoster (HZ) reactivation at least once in their lifetime (3), highlighting the need for efficient control of this disease.

The mechanism of protection from HZ reactivation is unclear, but cellular mediated immunity (CMI) has been shown to be integral to HZ prevention. VZV-antibody levels do not decline with age, but VZV-CMI has been observed to begin declining as early as age 35. Additionally, HZ severity has been clinically observed to be associated with a reduced magnitude of effector and effector memory VZV specific T cells (25).

Three vaccines are currently licensed in the United States for use in preventing VZV associated diseases. Varivax, a live attenuated vaccine for varicella immunity was first licensed in in the United States in 1995 and became a part of the recommended childhood vaccine schedule in 2005. In most cases, two doses are recommended in childhood (3). Two vaccines have been licensed for use in the United States to protect against Herpes Zoster (HZ): Zostavax, a live attenuated vaccine derived from the oka virus strain, and Shingrix, a vaccine based on recombinant protein vaccination. Zostavax was licensed in 2006 for people 50 years and older (3). The efficacy of Zostavax in preventing HZ appears to wane with age, from 70% efficacy in ages 50 – 59 to a mere 18% efficacy in vaccinees 80 years old or older (25, 27).

The Shingrix vaccine was recently licensed for adults 50 years and older and efficacy trials in adults have demonstrated protection regardless of age, with 91% efficacy observed in vaccinees 80 years or older (19). The Shingrix efficacy at older ages is yet to be fully understood, and additional studies dissecting the responses triggered by this vaccination are important for developing successful vaccination against other pathogens in the elderly population.

The recombinant VZV gE protein is co-formulated with the adjuvant system to induce both a strong CD4+ T cell and gE-specific antibody response (4, 19). VZV-specific CD4+ T cell responses are associated with positive vaccine outcomes (15, 42, 48, 51). The reasons for the remarkable difference in Shingrix efficacy may involve a capacity for eliciting a stronger and/or more focused response against the gE antigen, a broader epitope repertoire or an intrinsic difference in the quality and durability of the responses elicited. Several studies have indicated changes in the quality of the specific CD4+ T cell response towards a predominantly and persistently polyfunctional response after vaccination (6, 19, 26).

While studies have been conducted to assess the immunogenicity of different VZV proteins (24), and the immunological responses to VZV vaccinations have been described elsewhere (23, 28, 48, 49), there is still little known regarding the epitopes recognized by T cells after VZV vaccination. Identifying immunogenic epitopes and designing tetramers specific to VZV epitopes will allow further characterization of the observed polyfunctional response in Shingrix vaccinees and may help establish correlates of protection for HZ patients.

Here we assess the repertoire of CD4+ T cell responses induced by Shingrix vaccination and define a set of epitopes broadly recognized in these vaccinees. Further, we perform both predictive binding methods and reductive HLA restriction assays with identified VZV-specific CD4+ T cell epitopes to identify potential tetrameric reagents and immuno-monitoring strategies to address the mechanism of cell-mediated immunity and effective vaccination strategies.

Results

Recruitment of childhood exposure, post-shingles, and Zostavax and Shingrix vaccinee donor cohorts

To characterize CD4+ T cell responses following Shingrix vaccination we enrolled 18 Shingrix vaccinees, further sub-classified depending on their status before Shingrix vaccination as: Shingrix only (10), Shingrix after shingles (2), Shingrix after Zostavax (5), and triple conditions (1). To put our observations in a broader context of

other HZ related conditions, we also enrolled three independent cohorts of donors to represent individuals with childhood exposure (n=18), Zostavax vaccinees (n=18), and clinically diagnosed shingles with lack of vaccination (in childhood or otherwise) (n=18). The childhood exposure cohort encompassed donors who, based on date of birth, did not receive childhood vaccination and were likely to have experienced clinical chickenpox; as such they represent the natural exposure to VZV and never experienced shingles. A total of 72 donors participated in this study. Ethnicity, gender, average age, and time from most recent clinical event (Shingrix or Zostavax vaccination or HZ reactivation) are summarized in **Table 1**.

General features of VZV responses in the various cohorts.

To measure immune responses in the four cohorts we utilized various pools of peptides of different origin. In the case of the gE protein, 123 peptides (15-mers overlapping by 10) spanning the Dumas strain gE protein (Dumas sequence is 100% homologous with the Oka strain used in Zostavax) were arranged into a single "megapool", and then into 12 smaller mesopools of 10 or 11 peptides for subsequent epitope identification and deconvolution studies.

An additional 302 peptides from the remaining non-gE proteins, derived from the entire VZV proteome, were investigate to identify CD4+ T cell reactivity outside the gE protein. These included 44 epitopes from the published literature curated in the Immune Epitope Database (IEDB), and 258 predicted HLA class II dominant epitopes from the Dumas strain sequence (41). These peptides were arranged in two different megapools, one corresponding to structural proteins (S, excluding gE), and the other to the non-structural proteins (NS), as previously described (14). These peptide pools were used to characterize responses ex vivo in an Activation Induced Marker (AIM) assay (8) by analyzing the combined membrane markers for expression of CD137 (41BB) and OX40 molecules within the T cell's CD4+ compartment (**Fig 1**).

Strong responses to NS proteins were noted in the case of the childhood exposure and Shingles cohorts ($p=0.0004$ and $p<0.0001$, respectively, when compared to the unstimulated (DMSO) control with a non-parametric, paired Wilcoxon test). These responses were higher than those observed when S proteins were considered ($p= 0.0040$ and 0.0002 for childhood exposure and Shingles cohorts, respectively), with a trend towards S protein induced responses being actually lower in shingles subjects compared to non–shingles subjects (**Fig 1A).**

When the responses of the two vaccinated cohorts were examined, Zostavax and Shingrix vaccinees were found to be associated with significant responses to the various peptide pools. As expected, responses in Shingrix

vaccinees were remarkably vigorous towards gE (Shingrix: GeoMean = 0.18, $p < 0.0001$ compared to the unstimulated (DMSO) control) (**Fig 1B)**. These results confirmed that Shingrix vaccination induces a strong CD4+ T cell response directed against the gE antigen.

Immunodominant gE regions recognized specifically by Shingrix donors

Next, we defined the gE epitopes recognized in the Shingrix vaccinee cohort described above (**Table 1**), and performed donor HLA typing (**Supplemental Table 2)** to enable determination of the HLA restriction of any responding epitope. As we wanted to ensure that our cohort was representative of the general population, we compared the frequencies of the main HLA class II alleles observed in our cohort with those observed in our repository of over 3,500 donors (**Fig 2A**). This repository is inclusive of donors from a variety of clinical studies and representative of a diverse set of ethnicities and populations, ranging from the USA, South and Central America, Asia, South Africa and Europe, and therefore is a reasonable representation of the general population, with the further advantage of being HLA typed using the same methodology. Of the 28 different HLA class II alleles with phenotypic frequencies >10% in our cohort, 20 (71%) are also present in the general population with frequencies >10%. Additionally, 24 of the 27 (89%) alleles included in a reference panel of the most common and representative class II alleles in the general population (16) are also present in the current cohort (**Fig 2A**).

To identify individual epitopes, PBMCs were expanded with the gE MP for 14-17 days. On day 14, the gE megapool and mesopools were tested for cytokine reactivity. Positively identified pools were further deconvoluted on day 17, where the individual peptides of the positive pool were tested for cytokine reactivity. On both days 14 and 17, reactivity was assessed using a IFNγ, TNFα and IL-5 FluoroSpot assay (**Fig 2B**). A full list of the epitopes identified in this study has been submitted to the IEDB (http://www.iedb.org/ 1000857) and available in **Supplemental Table 1**.

Overall, we identified 89 epitopes recognized in at least one donor. When the response frequency and magnitude data were mapped to the gE sequence several immunodominant regions were identified (**Fig 2C**). Responses were found to be dispersed throughout the protein, with the exception of the 110 N-terminal residues, which were recognized poorly, if at all.

Comparison with the repertoire of epitopes recognized in different donor cohorts

The data presented above in **Fig 1**, indicate that Shingrix vaccinees have a stronger gE response as compared to other cohorts of VZV exposed/vaccinated individuals, and several epitopes were recognized per donor. (**Fig 2C**).. Those two points raised the question whether the higher Shingrix response was due to a stronger response to a similar number of epitopes, or responses of similar magnitude directed against a larger number of epitopes. To address this, we conducted pilot deconvolution experiments in two donors from the Zostavax cohort, and three donors from the childhood exposure cohort, as summarized in **Fig 3.**

First, we inspected the overlap in repertoires between the Shingrix and Zostavax/childhood exposure donors. Of the 89 epitopes identified in the Shingrix cohort, 42 (47%) were recognized also in the Zostavax/childhood exposure samples. Conversely, only 5 (5%) of the peptides not recognized in Shingrix vaccinees were recognized in Zostavax/childhood exposure samples ($p>0.0001$ by the Chi-squared test). This indicated that the epitope repertoire recognized in the Shingrix and Zostavax/childhood exposure donors is similar in nature.

Overall, in the Shingrix cohort the average donor responded to 18±10 epitopes, with a magnitude of 2041±1673 SFC/10^6 PBMC per response. By contrast, the Zostavax/childhood exposure donors responded to 10 ± 8 epitopes, with a magnitude of 1353±1080 SFC/10^6 PBMC per response. (**Fig 4**). Thus, although these differences are not significantly, Shingrix donors tend to have a broader epitope repertoire and responses of higher magnitude compared to the Zostavax/childhood exposure cohort.

Breadth and magnitude of gE-specific CD4+T cells

As mentioned above, of the 123 peptides were recognized in at least one Shingrix vaccinee, while only 34 were not recognized (**Fig 4A**) at all. 26 and 19 epitopes were recognized in only one or two donors, respectively, while the remaining 44were recognized in 3 or more. Similarly, if the response magnitude is considered, the top 34 epitopes are found to account for 80% of the total response (**Fig 3B**).

Table 3 lists these top 34 epitopes, representing those eliciting a response in four or more donors, and details the number of positive identifications, sum of SFC for peptide across donors, percentage (%) of total SFC, cumulative SFC, and response frequency (RF; see Methods) scores >0.1. For each epitope, the table shows position, sequence, total SFC, % of total SFC attributed to that peptide, and the number of donors recognizing it. Overall, these data indicate that while Shingrix vaccinees are associated with a broad and diverse repertoire, a limited number of epitopes are dominant and account for the majority of responses.

HLA-binding capacity of dominant epitopes

To characterize the dominant epitopes in more detail we conducted HLA/epitope binding assays, using purified HLA molecules in vitro, as described in the Methods. **Table 4** presents an analysis of a set of 19 common HLA class II alleles selected on the basis of worldwide frequencies (16). Purified HLA molecules were obtained as described in the methods section and binding capacity was evaluated using IC50 nM. 50% of tested epitopes bound to 7 or more alleles, with an average epitope recognizing 6.1±3.4 alleles. Actual binding data was generated for 28 of 34 of the epitopes in **Table 3**. Six additional epitopes (136, 146, 176, 221, 471 and 481) were not available when the binding assays were performed, and in those cases, HLA binding predictions were used instead using the NetMHCIIpan algorithm (1). The use of HLA binding predictions for those peptides was supported by the strong correlation detected for predicted versus actual measured binding (**Fig 6**) in the cases where both experimental and predicted binding values were determined.

Most of the epitopes were found to be promiscuous HLA class II binders. The epitopes recognized in four or more donors bound 6.1±3.4 alleles. By contrast, negative peptides (i.e. not recognized in any donor) were predicted to bind an average of 2.1±3.6 of the same panel of allelic variants.

HLA restriction determinations

Next, we determined HLA restrictions for selected epitopes by the use of single HLA transfected cell lines. Briefly, T Cell Lines (TCL) generated by 14 days in vitro re-stimulation with an individual peptide were tested in antigen presentation assays, where different cell lines expressing a single HLA class II molecule were used as APCs (31), as detailed in the Methods. Representative results are shown **Fig 5A-C.** When a TCL derived from a donor responding to the gE 446-460 peptide (sequence SHMEPSFGLILHDGG) was tested with single HLA transfected APC lines matching the HLA type of the donor, a good response was observed in the case of the HLA DPB1*0402 APCs. No response was noted for any of the other APC lines matching the donor's HLA type. Accordingly, the gE 446-460 peptide was determined to be DPB1*0402 restricted in this donor. Similarly, the gE 86-100 and 486-500 peptides (sequences NDYDGFLENAHEHHG and VEAVAYTVVSTVDHF, respectively) were identified as putatively restricted by DQB1*0302 and DRB1*0701 in other representative donors (denominated as "positive restrictions"). Conversely,

the HLA/epitope combinations tested in the same assays and found negative were denominated as "negative restrictions".

The results of the experiments are presented in **Table 5** and summarized in **Fig 5D**. Overall, we determined positive restriction for 38 peptides and 94 donor/peptide combinations (listed in **Supplemental Table S3**). Most positive restrictions were associated with the DRB1 and DQ loci, consistent with previous studies (36). Not all restrictions could be determined unequivocally, as APC corresponding to some of the HLA alleles were not available because of limitations in cell availability or other technical issues. In addition to testing as many of the peptides in **Table 3** as possible, we also tested certain peptides that were positive in only one or few donors, but yielded vigorous responses. **Table 5** presents a summary of determined restrictions, including sequence, percentage of the response, number of donors recognized and associated HLA restriction(s).

Predictive performance of different methodologies

The data set described above provided an opportunity to explore potential strategies to predict restrictions, without performing HLA restriction assays or performing in vitro HLA binding measurements. In a first determination, we explored the relative merit of four different methodologies to discern positive and negative restriction: the actual measured binding affinity, predicted binding affinity, percentile rank of predicted affinity, and the Response Frequency (RF) determination. The Area Under the Curve (AUC) values observed in the receiver operating characteristic (ROC) curves were used to quantify predictive efficacy.

As shown in **Fig 6A,** and as expected, actual measured binding was the most effective (AUC=0.7179), followed by predicted rank (AUC=0.6600). Since experimental binding determination is laborious and time consuming, for the subsequent analyses we focused on predicted rank as the most promising approach from the operational point of view. When the predictive efficacy was examined as a function of the different loci, we found that DRB1 (AUC= 0.7245; **Fig 6B)** and DQ (AUC=0.7542**; Fig 6C)** restrictions were predicted with reasonable accuracy. Conversely, the performance was much lower in the case of DP (AUC=0.5425; **Fig 6D**) and the DRB3/4/5 (AUC=0.6377; **Fig 6E**) loci, perhaps reflecting that these restrictions were relatively few and infrequent. Indeed, by considering only DRB1 and DQ loci as putative restriction elements, and utilizing 15th percentile rank as a threshold to define binding, we would have correctly called HLA restrictions for 39 of 94 instances, while also calling cases,

with 32 incorrect restrictions (false positives). In other words, utilizing those criteria we would be correct about 45% of the time and capture about 40% of the epitope restrictions.

Discussion

Here we report a detailed characterization of the epitope targets of the CD4 response of human subjects vaccinated with Shingrix. The results will enable mechanistic studies of vaccine efficacy, provide an example of a systematic analysis of epitope responses to a vaccine antigen, and have some methodological implications for future analyses and HLA restriction determinations.

Shingrix has shown increased efficacy in elderly and other groups as compared to the Zostavax vaccine (19, 42, 54). The reasons for this remarkable difference may involve intrinsic differences in the quality and durability of the responses elicited, also perhaps because of inclusion of the $AS01_b$ adjuvant. We believe that our results will facilitate mechanistic studies to directly address this point, by enabling HLA-class II tetramer studies and epitope pool-based isolation of reactive T cells.

In terms of enabling tetramer studies (9, 33), our results identified a total of 38 HLA/epitope experimentally defined restriction, which allow to cover an overwhelming majority of the human population, irrespective of ethnicity, and thus overcome a limitation of studies that use tetrameric reagent to capture and study antigen specific T cell, namely the availability of reagents covering few alleles, and typically those frequent in Caucasian ethnicities. Our results also enable an alternative approach, namely the use of pools of experimentally defined epitopes, as “bait” to capture and characterize antigen specific T cells (30). Tetrameric reagents incorporating these epitopes may inform if and how the recognized epitope affects polyfunctionality. Several studies have started to address these issues. Liang et al (24) reported that CD4+ T cells respond to different VZV ORFs, and that response increases after Zostavax vaccination. Zostavax- specific CD4+ T cells have a mixed Th1/Th2/Th21 phenotype (28). Increases in Tfh (and IL-21 in CD4s`in general) and mixed Th1/Th2 response with both IFNγ and IL-4 release were reported from PBMCs (28). Levin et al (26) reported the profiles and magnitude of Th1 T cell memory responses differentiate Shingrix and Zostavax., our study experimentally defined 38 HLA/epitope restrictions, which allow to cover an overwhelming majority of the human population, irrespective of ethnicity. This set thus allows to overcome a limitation of studies that use tetrameric reagents to capture and study antigen specific T cell, namely that the tetrameric reagents generally available only cover limited allelic diversity, and are typically inclusive only of those alleles frequent in Caucasian populations. Our results also enable an alternative approach, based on the use of pools of experimentally defined epitopes as “bait” to capture and characterize antigen specific T cells (30).

Tetrameric reagents incorporating these epitopes may inform if and how specific epitopes affect polyfunctionality. Several studies have started to address these issues. Liang et al. (24) reported that CD4+ T cells respond to different VZV ORFs, and that the response increases after Zostavax vaccination. Zostavax- specific CD4+ T cells have a mixed Th1/Th2/Th21 phenotype(28). Increases in Tfh (and IL-21 in CD4s, in general) and a mixed Th1/Th2 response with both IFNγ and IL-4 release were reported from PBMCs (28). Levin et al. (26) reported that the profiles and magnitude of Th1 T cell memory responses differentiate Shingrix and Zostavax.

Shingrix vaccination unsurprisingly induces a vigorous gE-specific response, in agreement with previous studies (13, 26, 43), together with a significant response against non-structural proteins, followed by other structural proteins. When we compared the responses across Zostavax vaccinees, childhood exposed and HZ patients were all found to have a similar response profile, with the strongest responses towards Non-structural proteins. This suggests also that the pattern of VZV specific CD4+ T cell protein immunodominance after natural exposure is in general against non-structural proteins; Zostavax vaccination mirrors the natural infection profile, while Shingrix skews the natural protein immunodominance toward gE. This is also in contrast with the heterogenic pattern of CD4+ T cell reactivity observed in young adults after chickenpox vaccination, suggesting that the priming with the natural infection might contribute to the reduced response against Zostavax in the elderly (14, 48).

We positively identified a total of 89 epitopes recognized across 18 Shingrix donors. Of these, the 34 most responsive capture 80% of the total (SFC) response against all identified epitopes. Thus, while Shingrix vaccinees are associated with a broad and diverse repertoire, a limited number of epitopes are dominant and account for the majority of responses. These epitopes will help build an immunogenic profile of VZV, as currently only 25 VZV gEv-derived T cell epitopes recognized in human have been reported in the published literature, as captured by the IEDB. Of the top 34 epitopes identified in our study, 7 (20%) nest or overlap with previously identified epitopes, while the remaining 27 (80%) are novel.

The results in terms of epitope breadth and coverage can be interpreted in the context of other studies from our laboratories that have used similar methodology to define the epitope repertoire of different antigens by scanning with sets of overlapping peptides. These studies included mycobacterial (29), pollen (38), cockroach (37) anddust mite allergens (20), and pertussis (37) and tetanus (7). The pattern of 20-30 epitopes encompassing the vast majority of the responses is generally applicable, and in this respect the gE antigen does not present unusual characteristics that might explain the remarkable vaccine efficacy.

Our results indicate that Shingrix vaccination might result in a marginally broader repertoire as compared to Zostavax and natural exposure, and a somewhat higher magnitude of responses. Indeed, broader responses have been invoked as a correlate of protection in the case of HCV and other viral infections (22, 44, 50). However, in the case of Shingrix versus Zostavax differences in the epitope breadth were not statistically significant and any difference should also be interpreted with caution, since the Shingrix samples were generally obtained closer to vaccination as compared to the Zoster cohort.

Finally, our data, in line with previous studies (30, 31) validates the use of single HLA transfected target cells as a valid methodology to experimentally determine HLA restrictions. The methodology is powerful and accurate, but somewhat laborious. In this context, our data set also allowed to evaluate the efficacy of three different methodologies to predict HLA restrictions in absence of experimental data. We conclude that using the percentile rank method is the best tool to predict restrictions for the DRB1 and DQ loci. Using a 15th percentile cut-off, we were able to accurately predict 45% of epitope/HLA combinations for DRB1 and DQ alleles identified in our dataset. This can help narrow down targets of investigation prior to performing assays and increase the speed and coverage of identifying HLA/epitope combinations.

Materials and Methods

Study subjects, PBMCs isolation, and HLA typing

The Shingrix vaccine cohort consists of healthy donors; adult male and non-pregnant female volunteers, age 50 and over, that were enrolled after receiving Shingrix vaccination (n=18) under the LJI program VD-184. PBMC derived from Shingrix cohorts were processed at LJI by density-gradient sedimentation using Ficoll-Paque (Lymphoprep; Nycomed Pharma, Oslo, Norway). Isolated PBMC were cryopreserved in heat-inactivated fetal bovine serum (FBS; HyClone Laboratories, Logan UT), containing 10% dimethyl sulfoxide (DMSO) (Gibco) and stored in liquid nitrogen until use in the assays.

HLA typing was performed by an ASHI-accredited laboratory at Murdoch University (Western Australia) for Class II (DRB1, DRB3/4/5, DQA1/DQB1, DPB1) as previously described (17, 18, 30). **Table 1** describes general cohort characteristics including ethnicity, race, gender, age. **Supplemental Table 2** lists the HLA-typing of the Shingrix vaccinees.

Peptides, Mesopools, and Megapool

Overlapping 15-mers by 10 were derived from gE protein of the Dumas reference sequence (NC_001348.1) available in the Viral Expasy Database (https://viralzone.expasy.org/), for a total of 123 peptides. Peptides were synthesized as crude material (A&A, San Diego, CA) and resuspended in DMSO at a concentration of 20 mg/mL.

Part of the peptide synthesis was either pooled in small mesopools, containing 10 peptides each, or pooled together with all the gE peptides and sequentially lyophilized as previously described to generate a gE Megapool(2).

gE epitopes identified in this study were mapped to the gE Dumas reference sequence (NC_001348.1) to identify immunodominant regions, using the Immunobrowser tool (12).Part of the peptide synthesis was either pooled in small pools, containing 10 peptides each, or pooled together with all the gE peptides, and sequentially lyophilized as previously described to generate a gE Megapool(2). gE epitopes identified in this study were mapped to the gE Dumas reference sequence (NC_001348.1) to identify immunodominant regions using the IEDB's Immunobrowser tool (12).

Activation Induced Marker (AIM) Assay

Cells were cultured in the presence of either gE, S, NS MPs (2ug/mL), DMSO (2%), or phytohemagglutinin (10ug/mL) (PHA) for 22-24 hours. After stimulation, cells were stained with surface markers for 30 minutes at 4°C**.** Detailed information for all the antibodies used for flow cytometry experiments in this study can be found in **Table 6.** Surface marker proteins were quantified via flow cytometry (LSRII, BD Biosciences) and analyzed using FlowJo software version 10.5.3 (TreeStar Inc., Ashland, OR). The gating strategy is schematically represented in (**Figure 8**). Statistical analyses were performed using Graphpad Prism v.8 (San Diego, CA). Responses of paired samples across different stimuli were tested using a Wilcoxon matched paired test.

Epitope identification by FluoroSpot

PBMCs were expanded in the presence of gE Megapool (MP) at a concentration of 1µg/mL for 14 days. After culturing, cells were collected and restimulated in triplicate at a density of $5x10^4$ cells/well with gE MP (1µg/mL), smaller mesopools containing 10 peptides each (1µg/mL), PHA (10µg/mL), and DMSO (0.1%) in 96-well plates previously coated with anti-cytokine antibodies for IFNγ, TNFα, and IL-5 (mAbs 1-D1K, MT25C5, TRFK5, respectively; Mabtech, Stockholm, Sweden) at a concentration of 10µg/mL.

After 20hr incubation at 37ºC, 5% CO2, cells were discarded and FluoroSpot plates were washed and further incubated for 2hr with cytokine antibodies (mAbs 7-B6-1-BAM, 5A10 biotinylated, MT20D9-WASP, respectively; Mabtech, Stockholm, Sweden). Subsequently, plates were washed again with PBS/0.05% Tween20 and incubated for 1hr with fluorophore-conjugated antibodies (Anti-BAM-490, SA-550, and Anti-WASP-640). Computer-assisted image analysis was performed by counting fluorescent spots using an AID iSPOT ELISPOT reader (AIS-diagnostika, Germany).

Each mesopool was considered positive compared to the background based on the following criteria: 100 or more spot forming cells (SFC) per 10^6 PBMC after background subtraction for each cytokine analyzed, a stimulation index (S.I.) greater than 2, and statistically different from the background ($p<0.05$) in either a Poisson or T test.

Mesopools eliciting the strongest cytokine response were deconvoluted at day 17 into the corresponding peptides at a concentration of 10ug/mL, and analyzed using the same criteria applied for the mesopools.

Single HLA transfected cell lines

Single HLA transfected fibroblast (DAP.3) or B lymphocyte (RM3) cell lines were generated and maintained as previously described (30, 31). Based on the HLA typing of the donor, we assigned the HLA transfected cells considering only matches for the HLA class II DR, DP and DQ beta chains. When multiple cell lines correspond to the same beta chain, the alpha chain was matched based on the typing as well.

Short term T Cell cultures (TCL) were set up using donor PBMC cultured individually in the presence of single epitopes previously identified in the epitope identification experiments at 1ug/mL concentration for 14 days. At day 13, APC lines fibroblast-derived were induced with butyric acid (100 µg/mL) to favor HLA-expression on the cell membrane.

The following day, all APC lines were harvested, and viability (all >75%) was determined using Trypan Blue. Each APC line was pulsed individually with peptide at a concentration of 10ug/mL for 1hr at 37 ºC, 5% CO_2. TCLs were stimulated in triplicate with either a peptide-pulsed APC line, APC line alone (as a control), peptides (1µg/mL), PHA (10µg/mL), or DMSO (0.1%) in 96 well plates previously coated with anti-cytokine antibodies for IFNγ, TNFα, and IL-5 at a concentration of 10µg/mL. FluoroSpot assays and analysis criteria were carried out as described above for the epitope identification experiments. Computer-assisted image analysis was performed by counting fluorescent spots using Mabtech's IRIS FluoroSpot and ELISpot reader (Mabtech, Sweden).

HLA binding assay

Classical competition assays to quantitatively measure peptide binding to purified class II MHC molecules were performed as previously described (46). Briefly, a high affinity radiolabeled peptide was co-incubated at room temperature or 37C with purified MHC, a cocktail of protease inhibitors, and various concentrations of unlabeled inhibitor peptide. Following a two-day incubation, MHC bound radioactivity was determined by capturing MHC/peptide complexes on Ab coated Lumitrac 600 plates (Greiner Bio-one, Frickenhausen, Germany), and measuring bound cpm using the TopCount (Packard Instrument Co., Meriden, CT) microscintillation counter. The concentration of peptide yielding 50% inhibition of the binding of the radiolabeled peptide was calculated. Under the conditions utilized, where [label]<[MHC] and IC50 ≥ [MHC], the measured IC50 values are reasonable approximations of the true Kd values. Each competitor peptide was tested at six different concentrations covering a 100,000-fold range, and in three or more independent experiments. As a positive control, the unlabeled version of the radiolabeled probe was also tested in each experiment.

RF score calculation and HLA epitope prediction.

T cell epitope prediction analyses, as well as Relative Frequency (RF) score calculations, were performed using IEDB analysis tools (11). The relative frequency (RF) score is a combined metric that accounts for the positivity rate (how frequently an epitope elicits a positive response) and the number of independent assays. Specifically, RF = [(r – sqrt(r)]/t, where r is the total number of responding donors and t is the total number of donors tested (2). The RF score has been calculated using RATE tool (39, 40). HLA class II binding predictions were performed following two different strategies: one using NetMHCIIpan 3.2 (21) only and extracting IC50 predicted values, and the other one using the IEDB recommended 2.22 methodology and extracting rank percentile values. The IEDB recommended 2.22 methodology is based on a combination of seven prediction algorithms with priority on the consensus method (53), which includes comblib (45), SMM (35) and NN (34), followed by NetMHCIIpan 3.2 (21).

Data Availability

A complete list of the epitopes identified in this study has been submitted to the IEDB (http://www.iedb.org/ 1000857).

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H. Voic, R. de Vries, P. Rubiro, and E. Moore performed experiments. R. de Vries and J. Sidney assisted in the bioinformatics analyses. S. Mallal and E. Philips performed HLA typing. B. Schwa provided samples and sample information. A.Grifoni and H.Voic reviewed the data and planned the experimental strategy. H. Voic, A. Grifoni, A.

Sette and D. Weiskopf conceived and directed the study and wrote the manuscript. All the authors have critically read and edited the manuscript.

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Figures Legends

FIG 1. VZV-specific CD4+ T cell reactivity in natural exposure, severe disease and vaccination. Frequency of CD137+OX40+CD4+ T cells (see **FIG 8** for gating strategy) was assessed for donor/MP stimulation combinations and compared to a paired DMSO negative control. A) Childhood exposure (black circles, n=18) and Shingles cohorts (blue circles, n=18). B) Shingrix (orange circles, n=18) and Zostavax (grey circles, n=18) cohorts. Data are expressed as the geometric mean and geometric standard deviation (SD). All reported statistical analyses have been performed using non-parametric, paired Wilcoxon test.

FIG 2. **Epitope mapping of Shingrix vaccinees.** A) HLA frequency of the Shingrix cohort (n= 18) compared to general population HLA frequency (n=3,500). B) Experimental scheme; representative donor with the mesopool reactivity at day 14 and peptide deconvolution of one of the positive mesopools at day 17 and relative FluoroSpot images for IFNγ, IL-5 and TNFα. C) Immunodominant regions of the gE protein showing the total magnitude of responses (solid lines) and the number of responding subjects (dotted lines).

FIG 3. Shingrix vaccinees are associated with a broad CD4+ T cell response. A) The number of responding donors are plotted against the number of epitopes recognized. B) The total number of epitopes recognized are plotted against the percentage (%) of total magnitude of response (sum of total SFC of total responding donors).

FIG 4. Shingrix patients show a trend for recognizing more epitopes and producing a stronger response. A) Number of recognized epitopes (black) over the non-recognized ones (grey) in Shingrix, Zostavax/childhood exposure or both (****P<0.0001, Chi-square test). B) Number of recognized epitopes per donor-cohort (ns; p= 0.3095) C) Magnitude of responses per donor-cohort (ns; p=0.7743). (B-C) Mann-Whitney test was applied for statistical comparisons across cohorts.

FIG 5. Donor HLA/epitope restrictions identified by HLA restriction assay. (A-C) Representative examples showing determination of HLA restriction for three epitopes in three donors. D) Summary of positive and negative epitope/HLA restrictions identified.

FIG 6. Correlation of predicted versus experimentally measured binding. Experimental HLA binding (x-axis, IC50 nM) compared to the predicted HLA binding (NetMHCpan 3.2, IC50nM,(1)). Pearson correlation was applied (r = 0.9689, p<0.0001).

FIG 7. ROC curves of measured and predictive systems to infer HLA restriction. A) Comparison of different methodologies. B) HLA prediction based on rank percentile scores and as a function of the different HLA class II loci analyzed.

FIG 8. Representative Gating Strategy.

Table 1. General characteristics of donor cohorts.

Cohort	Donors	Caucasian (%)	Female (%)	Age (years; Average ± SD)	Time from exposure (days; average± SD)
Childhood Exposure	18	61	8	51 ±17	NA
Shingles	18	44	13	44 ±12	1686±2305
Shingrix	18	72	10	62 ±8	123 ±92
Zostavax	18	100	12	69 ±7	1849±1112

Table 2. HLA class II allele frequency in Shingrix cohort compared to the general population.

	Allele	Phenotype frequency	
		Cohort	General population
DR	DRB1*01:01	11.8	9.1
	DRB1*01:02	11.8	3.6
	DRB1*03:01	11.8	11.1
	DRB1*03:02	5.9	1.8
	DRB1*04:01	11.8	6.2
	DRB1*04:02	5.9	1.7
	DRB1*04:05	11.8	2.7
	DRB1*07:01	47.1	29.0
	DRB1*08:01	5.9	1.2
	DRB1*09:01	11.8	3.7
	DRB1*11:01	5.9	8.8
	DRB1*13:01	11.8	9.3
	DRB1*13:03	5.9	1.3
	DRB1*14:01	11.8	2.3
	DRB1*15:01	23.5	18.4
	DRB3*01:01	23.5	14.6
	DRB3*02:02	23.5	33.5
	DRB4*01:01	70.6	55.1
	DRB5*01:01	23.5	25.0
DQ	DQB1*02:01	11.8	12.3
	DQB1*02:02	35.3	18.7
	DQB1*03:01	29.4	29.0
	DQB1*03:02	11.8	19.8
	DQB1*03:03	17.6	12.0
	DQB1*04:02	11.8	7.4
	DQB1*05:01	17.6	20.2
	DQB1*05:03	11.8	10.7
	DQB1*06:02	23.5	15.8
	DQB1*06:03	11.8	10.2
DP	DPB1*01:01	5.9	17.0
	DPB1*02:01	35.3	23.4
	DPB1*03:01	23.5	11.6
	DPB1*04:01	35.3	47.5
	DPB1*04:02	35.3	18.2
	DPB1*05:01	5.9	15.0
	DPB1*14:01	5.9	0.2
	DPB1*15:01	5.9	1.0
	DPB1*17:01	5.9	6.5

Table 3. Most Commonly recognized gE epitopes among Shingrix vaccinees.

Position	Sequence	Positive/ tested	Total SFC	% total SFC	Cum. SFC	RF score
281	IEPGVLKVLRTEKQY	14/17	51227	5.5	5.5	0.603
191	IYGVRYTETWSFLPS	13/18	63696	6.8	12	0.522
186	APIQRIYGVRYTETW	12/18	32754	3.5	16	0.474
291	TEKQYLGVYIWNMRG	11/17	17568	1.9	18	0.452
421	AQRVASTVYQNCEHA	11/18	57444	6.2	24	0.427
356	TFSLAMHLQYKIHEA	10/17	38384	4.1	28	0.402
286	LKVLRTEKQYLGVYI	10/17	33867	3.6	32	0.402
196	YTETWSFLPSLTCTG	10/18	32942	3.5	35	0.380
486	VEAVAYTVVSTVDHF	9/17	43390	4.6	40	0.353
476	YVFVVYFNGHVEAVA	8/17	30807	3.3	43	0.304
51	YEPYYHSDHAESSWV	8/17	17507	1.9	45	0.304
446	SHMEPSFGLILHDGG	8/18	27209	2.9	48	0.287
31	SVLRYDDFHIDEDKL	8/18	23577	2.5	50	0.287
341	FHMWNYHSHVFSVGD	7/17	19080	2.0	52	0.256
386	TCQPMRLYSTCLYHP	6/17	21414	2.3	55	0.209
496	TVDHFVNAIEERGFP	6/17	19377	2.1	57	0.209
141	DDRHKIVNVDQRQYG	6/17	16362	1.8	59	0.209
181	PFTLRAPIQRIYGVR	6/18	15142	1.6	60	0.197
26	NPVRASVLRYDDFHI	6/18	13123	1.4	62	0.197
36	DDFHIDEDKLDTNSV	6/18	9080	1.0	63	0.197
221	LKHTTCFQDVVVDVD	5/17	27263	2.9	65	0.163
336	PRGAEFHMWNYHSHV	5/17	16907	1.8	67	0.163
391	RLYSTCLYHPNAPQC	5/17	12968	1.4	69	0.163
146	IVNVDQRQYGDVFKG	5/17	7440	0.8	69	0.163
351	FSVGDTFSLAMHLQY	5/17	4745	0.5	70	0.163
416	TSPHLAQRVASTVYQ	5/18	6103	0.7	71	0.154
501	VNAIEERGFPPTAGQ	4/17	11977	1.3	72	0.118
491	YTVVSTVDHFVNAIE	4/17	10803	1.2	73	0.118
481	YFNGHVEAVAYTVVS	4/17	9727	1.0	74	0.118
136	PTLNGDDRHKIVNVD	4/17	8903	1.0	75	0.118
471	SLSGLYVFVVYFNGH	4/17	3957	0.4	75	0.118
41	DEDKLDTNSVYEPYY	4/18	21923	2.3	78	0.111
436	DNYTAYCLGISHMEP	4/18	9900	1.1	79	0.111
176	VEENHPFTLRAPIQR	4/18	5198	0.6	79	0.111

Table 4. Observed in vitro binding capacity of 19 common HLA alleles to epitopes commonly recognized among Shingrix vaccinees.. Predictive data is shown for italicized rows.*

			DQA1/B1				DRB1											DRB3/4/5			
Start	**Sequence**	**Bound***	**5:01/ 2:01**	**3:01/ 3:02**	**1:01/ 5:01**	**1:02/ 6:02**	**1:01**	**3:01**	**4:01**	**4:05**	**7:01**	**8:02**	**9:01**	**11:01**	**12:01**	**13:02**	**15:01**	**B3*01:01**	**B3*02:02**	**B4*01:01**	**B5*01:01**
26	NPVRASVLRYDDFHI	8	3623	2232	**65**	**110**	10732	9222	5950	11471	1964	-	**417**	-	8460	**454**	**2.8**	**494**	34700	**414**	**462**
31	SVLRYDDFHIDEDKL	5	**368**	2547	**9**	13217	-	3824	**178**	2410	-	36884	7252	-	-	1996	**13**	**495**	-	1140	16474
36	DDFHIDEDKLDTNSV	3	1314	29358	**586**	-	-	2960	1393	**189**	-	-	35428	-	-	18728	1555	1276	-	**155**	-
41	DEDKLDTNSVYEPYY	1	**705**	3321	14664	14865	10147	14990	8611	8484	-	34714	-	-	-	6424	3750	18708	-	7851	35316
51	YEPYYHSDHAESSWV	7	**249**	11453	1048	7333	**620**	10926	**21**	10156	2476	**972**	**150**	4873	-	-	**553**	**164**	10958	-	9457
136	*PTLNGDDRHKIVNVD*	*1*	*13356*	*16063*	*18153*	*####*	*4878*	***235***	*6667*	*9636*	*8633*	*8658*	*8278*	*4653*	*11337*	*1636*	*7104*	*1147*	*6835*	*3630*	*3540*
141	DDRHKIVNVDQRQYG	6	16483	-	-	36976	28740	1473	**934**	12276	20608	7017	**119**	13429	**358**	**976**	**952**	2208	8380	**170**	3539
146	*IVNVDQRQYGDVFKG*	*1*	*6345*	*11676*	*6380*	*9447*	*4206*	***159***	*6015*	*6701*	*8397*	*6221*	*5678*	*5966*	*5079*	*3388*	*4922*	*1957*	*11176*	*2153*	*5339*
176	*VEENHPFTLRAPIQR*	*5*	*4968*	*5186*	*7261*	*1746*	***246***	*3585*	*1975*	*2184*	*1209*	*1398*	***997***	***600***	*4161*	*1385*	*1933*	*7537*	*4013*	***769***	***156***
181	PFTLRAPIQRIYGVR	11	4367	-	14657	5264	**56**	17788	1421	5434	**845**	**48**	**60**	**949**	**593**	**55**	**21**	5565	**996**	**96**	**11**
186	APIQRIYGVRYTETW	8	**174**	10539	7410	15811	1403	11307	3525	6552	**183**	1567	**880**	5105	**194**	**684**	**5**	8514	11715	**447**	**117**
191	IYGVRYTETWSFLPS	11	**245**	3883	2471	8109	**102**	27309	**215**	**625**	**19**	15656	**13**	5158	1522	**174**	**108**	**211**	10142	**355**	**115**
196	YTETWSFLPSLTCTG	9	**634**	4134	**381**	8210	**20**	38807	**40**	**149**	**154**	2654	**49**	1259	1855	-	**574**	26572	19164	14325	**428**
221	*LKHTTCFQDVVVDVD*	*3*	***263***	*1227*	***851***	*1850*	*2141*	*1718*	*2097*	*1528*	*2659*	*6991*	*3029*	*9804*	*5953*	*2808*	*5264*	***547***	*9101*	*3230*	*9423*
281	IEPGVLKVLRTEKQY	11	19213	18164	-	2334	**82**	21908	**83**	**202**	9613	**223**	**447**	**95**	**464**	5947	**250**	-	**980**	**1.7**	**100**
286	LKVLRTEKQYLGVYI	10	**677**	8649	2165	6510	**197**	4210	**58**	1137	1265	**452**	**491**	1062	**260**	2171	**25**	9580	**882**	**1.3**	**837**
291	TEKQYLGVYIWNMRG	10	35859	1650	**958**	1071	**543**	-	**230**	**240**	**669**	2172	**602**	1159	-	10843	**0.87**	**933**	2633	**295**	**124**
336	PRGAEFHMWNYHSHV	5	-	-	2913	-	1067	-	**710**	3232	**845**	1008	**227**	4167	-	**703**	**2.1**	1997	4486	1307	1288
341	FHMWNYHSHVFSVGD	7	-	4505	1254	**30**	1120	-	**833**	1538	**2.5**	**104**	**4.2**	1213	-	15002	**6.7**	3858	-	1316	**809**
351	FSVGDTFSLAMHLQY	7	7174	4629	5112	**95**	1310	29944	4939	20004	**673**	**243**	**20**	**349**	13509	-	**26**	8016	-	**99**	6730
356	TFSLAMHLQYKIHEA	8	7477	-	**44**	3091	1221	-	1118	1928	**29**	**274**	**11**	**500**	13602	22315	**6.3**	1729	32786	**204**	**158**
386	TCQPMRLYSTCLYHP	3	-	-	1561	2895	**526**	-	1065	1495	1986	4466	**382**	29468	4733	-	**14**	3375	15526	2117	5306
391	RLYSTCLYHPNAPQC	2	12424	24467	1263	3646	2078	-	**431**	1640	8632	10285	2133	11791	-	28343	**452**	2515	3744	-	9370
416	TSPHLAQRVASTVYQ	8	10103	32230	-	**270**	**76**	-	**72**	**485**	**193**	2756	**128**	-	36480	1948	**99**	-	1911	**380**	2390
421	AQRVASTVYQNCEHA	1	6622	20639	-	4719	21756	-	5348	5717	12948	-	10260	-	-	1886	**260**	-	-	-	21713
436	DNYTAYCLGISHMEP	4	**904**	2657	**723**	3245	1782	-	1758	**124**	8313	1209	**164**	10985	-	-	1189	29400	-	7038	5268
446	SHMEPSFGLILHDGG	7	3667	11444	2527	**547**	**223**	5435	**176**	6685	**193**	1099	**12**	3601	-	**968**	**9.1**	1577	19700	3678	1015
471	*SLSGLYVFVVYFNGH*	*2*	*3316*	*6809*	*1382*	*1926*	***696***	*13342*	*4047*	*2121*	*1643*	*4501*	*1901*	*4324*	*2346*	*4869*	***811***	*7255*	*14123*	*3739*	*5531*
476	YVFVVYFNGHVEAVA	5	1152	5046	1251	1519	**134**	-	**195**	1017	2044	4550	**85**	15029	-	**138**	**1.3**	3018	12800	9167	10118
481	*YFNGHVEAVAYTVVS*	*4*	*1036*	*1303*	*3416*	***262***	***127***	*7849*	*1276*	*1553*	***283***	*2085*	***208***	*3879*	*2968*	*1445*	*2064*	*3488*	*4579*	*2657*	*1882*
486	VEAVAYTVVSTVDHF	10	**894**	2086	1586	2693	1108	31488	**72**	**53**	**96**	**696**	**148**	8329	-	**138**	**95**	1581	10737	**153**	**531**
491	YTVVSTVDHFVNAIE	10	**515**	**604**	**69**	16277	**388**	12518	**930**	**69**	**109**	3761	**243**	-	-	6708	**107**	8010	10222	**912**	4935
496	TVDHFVNAIEERGFP	11	**63**	**249**	**598**	**343**	**103**	1661	**63**	**369**	5244	1608	**230**	**724**	2987	6998	**433**	10694	7314	**219**	1666
501	VNAIEERGFPPTAGQ	3	**184**	6672	10872	9640	3732	-	6381	16505	-	-	12975	-	-	-	**455**	-	-	**872**	-

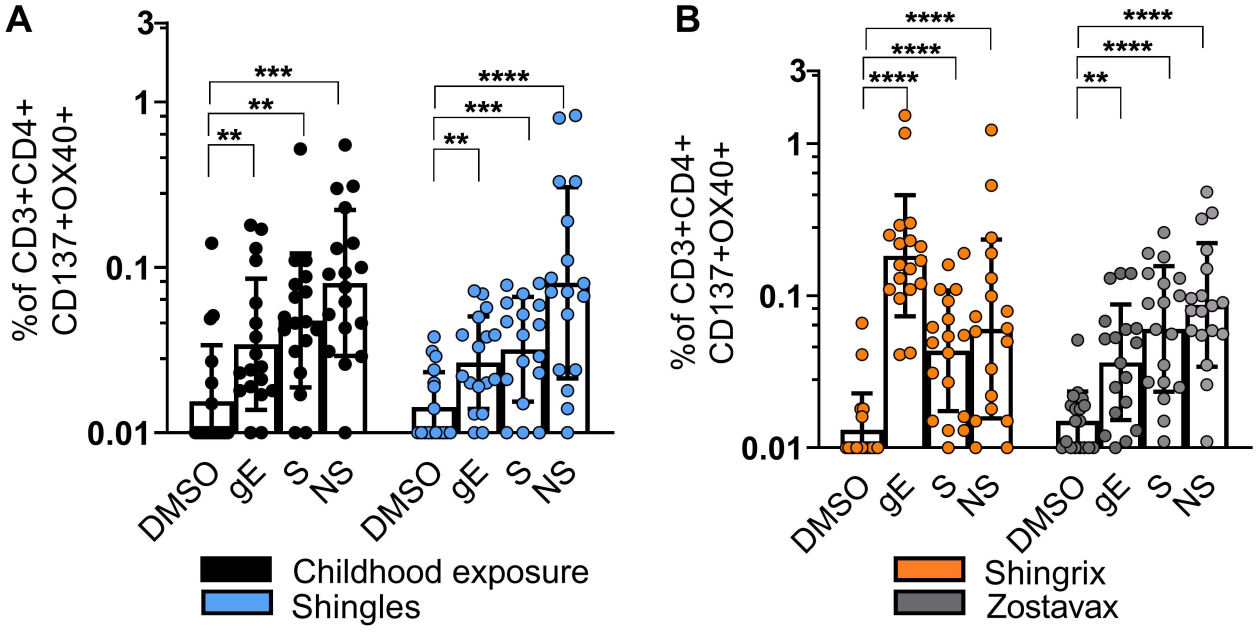
*number of bound peptides per each HLA allele with an IC50<1000nM. Dash represents IC50> 40000.

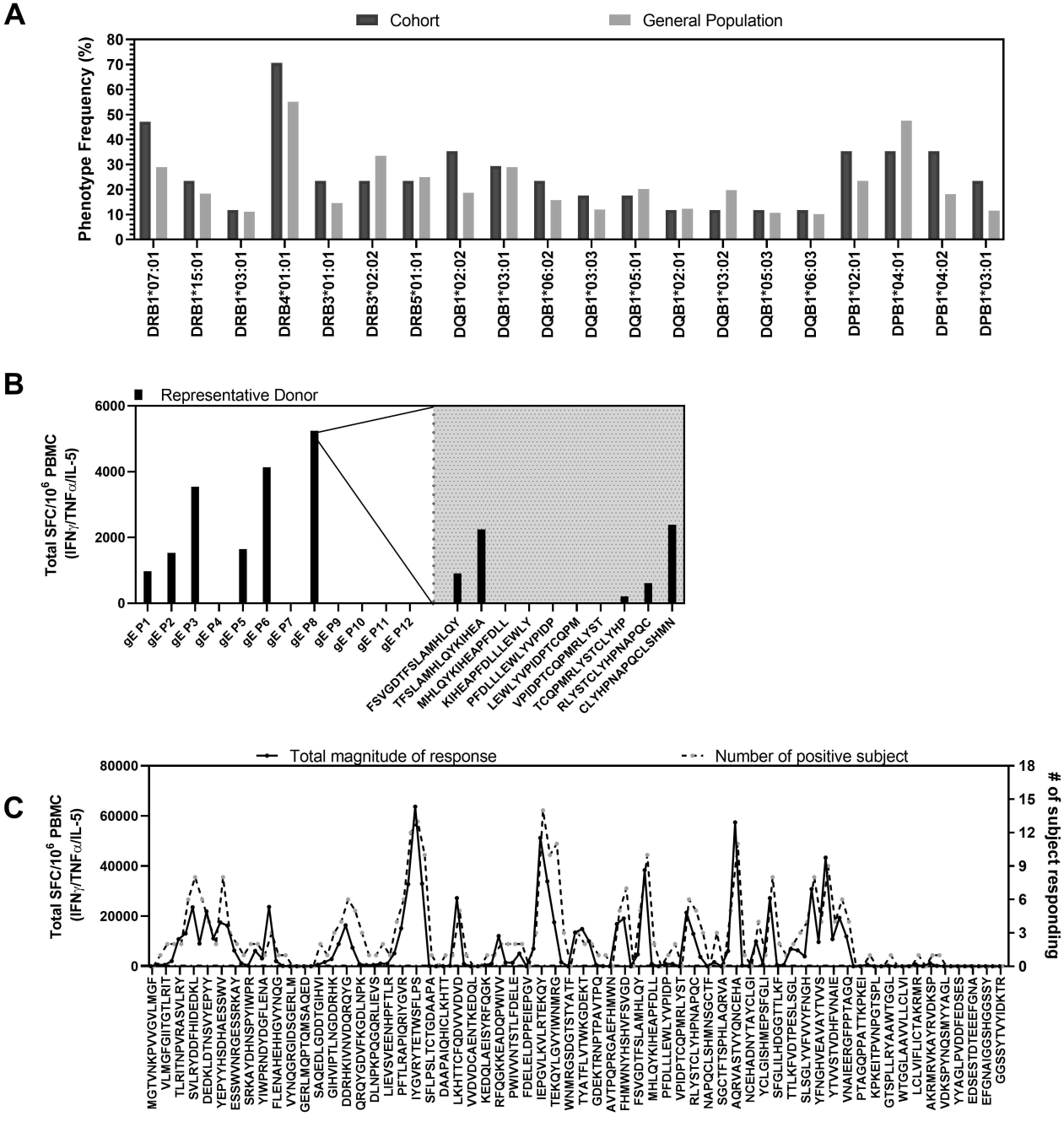
Table 5. Summary of determined epitope/HLA restrictions.

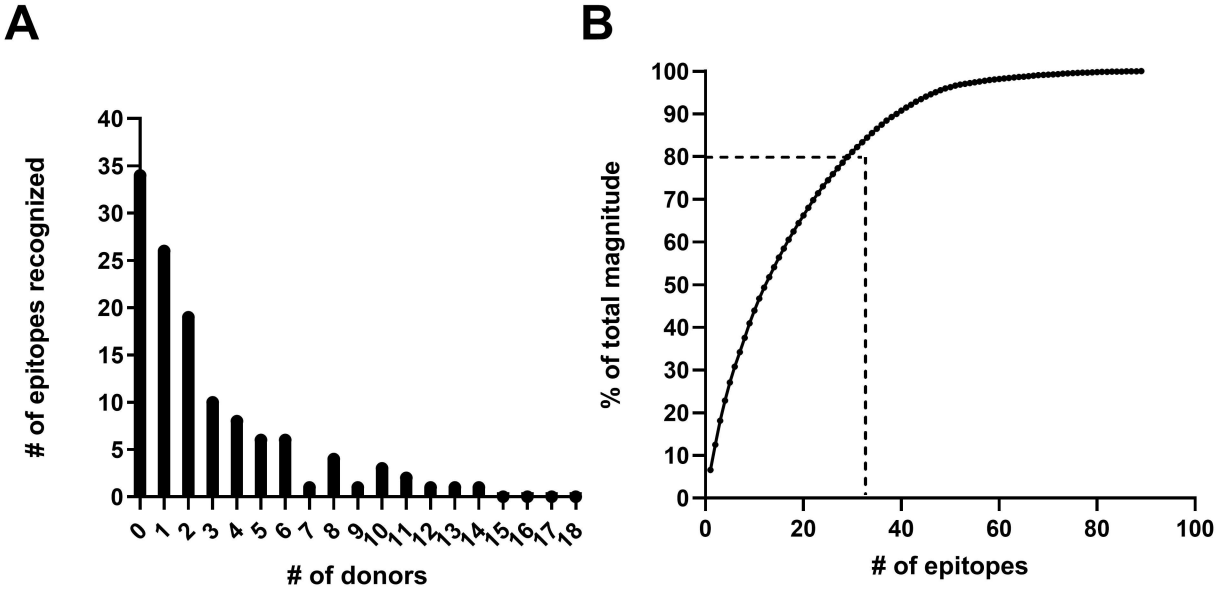
Sequence	Sum of SFC	Sum of % Response	Restricted Alleles
APIQRIYGVRYTETW	11446.67	1.59	DRB1*07:01, DRB3*02:02
AQRVASTVYQNCEHA	33653.33	4.67	DRB1*07:01, DPA1*01:03/DPB1*04:01, DRB1*15:01, DPA1*01:03/DPB1*02:01, DRB4*01:01, DPA1*03:01/DPB1*04:02, DRB5*01:01
DDFHIDEDKLDTNSV	600.00	0.08	DRB1*13:03
DEDKLDTNSVYEPYY	1406.67	0.19	DPA1*01:03/DPB1*02:01
DHNSPYIWPRNDYDG	386.67	0.05	DRB1*13:01
DNYTAYCLGISHMEP	24133.33	3.35	DRB1*09:01
ESSWVNRGESSRKAY	63200.00	8.76	DQA1*05:01/DQB1*03:01
FHMWNYHSHVFSVGD	966.67	0.13	DRB4*01:01, DPA1*01:03/DPB1*02:01, DQA1*03:01/DQB1*03:02
FLENAHEHHGVYNQG	4226.67	0.59	DQA1*04:01/DQB1*04:02, DQA1*01:01/DQB1*05:01
FSVGDTFSLAMHLQY	293.33	0.04	DRB1*09:01
HSDHAESSWVNRGES	16520.00	2.29	DQA1*05:01/DQB1*03:01, DRB1*13:01
IEPGVLKVLRTEKQY	11980.00	1.66	DRB1*04:05, DRB1*14:01
IQHICLKHTTCFQDV	1393.33	0.19	DPA1*03:01/DPB1*04:02, DQA1*01:02/DQB1*06:02
IVNVDQRQYGDVFKG	46026.67	6.38	DQA1*01:01/DQB1*05:01, DQA1*05:01/DQB1*02:01, DRB1*03:01
IYGVRYTETWSFLPS	33373.33	4.63	DRB1*07:01, DPA1*02:01/DPB1*05:01
LEWLYVPIDPTCQPM	3360.00	0.47	DQA1*01:01/DQB1*02:01
LGVYIWNMRGSDGTS	33386.67	4.63	DQA1*01:01/DQB1*05:01, DQA1*04:01/DQB1*04:02
LKHTTCFQDVVVDVD	35686.67	4.95	DQA1*01:01/DQB1*02:01, DQA1*01:01/DQB1*05:01
LKVLRTEKQYLGVYI	3833.33	0.53	DRB1*14:01
LVTWKGDEKTRNPTP	6893.33	0.96	DPA1*02:01/DPB1*05:01, DPA1*01:03/DPB1*04:01, DRB1*13:03
NDYDGFLENAHEHHG	6446.67	0.89	DQA1*04:01/DQB1*04:02, DQA1*03:01/DQB1*03:02
PRGAEFHMWNYHSHV	16120.00	2.23	DRB1*15:01, DPA1*01:03/DPB1*02:01
PTLNGDDRHKIVNVD	33353.33	4.62	DQA1*01:01/DQB1*02:01, DRB1*03:02, DRB1*03:01
RLYSTCLYHPNAPQC	4400.00	0.61	DPA1*01:03/DPB1*04:01
SHMEPSFGLILHDGG	50273.33	6.97	DRB1*07:01, DRB1*09:01, DQA1*05:01/DQB1*03:01
SVLRYDDFHIDEDKL	37026.67	5.13	DRB1*15:01, DQA1*01:01/DQB1*05:01
TCQPMRLYSTCLYHP	9213.33	1.28	DPA1*01:03/DPB1*04:01, DRB4*01:01
TEKQYLGVYIWNMRG	566.67	0.08	DRB1*03:02, DQA1*04:01/DQB1*04:02, DRB1*13:01
TVDHFVNAIEERGFP	7053.33	0.98	DQA1*05:01/DQB1*02:01
TYATFLVTWKGDEKT	44200.00	6.13	DPA1*02:01/DPB1*05:01
VDTPESLSGLYVFVV	986.67	0.14	DRB1*07:01
VEAVAYTVVSTVDHF	39460.00	5.47	DRB1*07:01, DQA1*05:01/DQB1*03:01, DRB3*02:02, DRB4*01:01
VEENHPFTLRAPIQR	29973.33	4.16	DQA1*01:02/DQB1*06:02
YCLGISHMEPSFGLI	24680.00	3.42	DQA1*01:02/DQB1*06:02
YEPYYHSDHAESSWV	11153.33	1.55	DRB1*04:02, DQA1*03:01/DQB1*03:01, DPA1*01:03/DPB1*04:01, DQA1*05:01/DQB1*02:01, DRB4*01:01
YFNGHVEAVAYTVVS	5700.00	0.79	DQA1*05:01/DQB1*03:01
YTETWSFLPSLTCTG	5573.33	0.77	DQA1*01:01/DQB1*05:01, DQA1*03:01/DQB1*03:02, DRB1*07:01
YVFVVYFNGHVEAVA	62426.67	8.65	DQA1*05:01/DQB1*03:01
Total	721373.333	100	

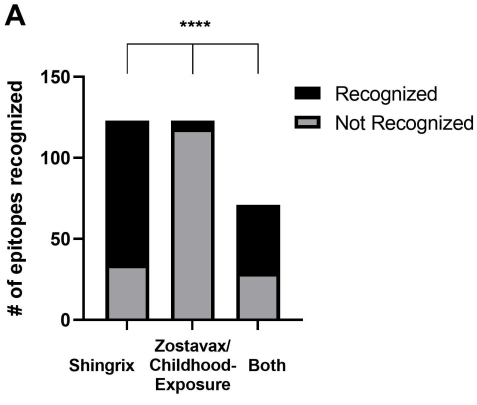
Table 6. AIM Flow Cytometry Panel. Antibody panel used in flow cytometry experiments to identify both magnitude and quality of CD4+ T Cell response.

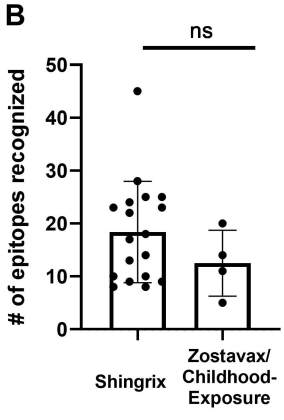
Antibody	Flourocrome	Volume (µl/well)	Vendor	Catalog	Clone
CD3	AF700	2	Biolegend	317340	OKT3
CD14	V500	1	BD	561391	M5E2
CD19	V500	1	BD	561121	HIB19
Live/Dead	ef506/Aqua	0.5	eBioscience	65-0866-18	--
CD8	Cy5.5	2	eBioscience	45-0088-42	RPA-T8
CD4	Pacific Blue	2	BD	558116	RPA-T4
OX40	PE-Cy7	2	Biolegend	350012	Ber-ACT35
CD137	APC	2	Biolegend	309810	4B4-1

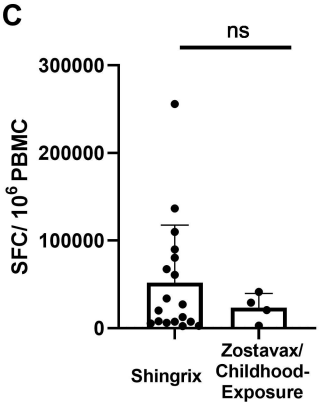

A
B
%of CD3+CD4+
CD137+OX40+
3
1
0.1
0.01
***
**
****
DMSO
gE
S
NS
Childhood exposure
Shingles
Shingrix
Zostavax

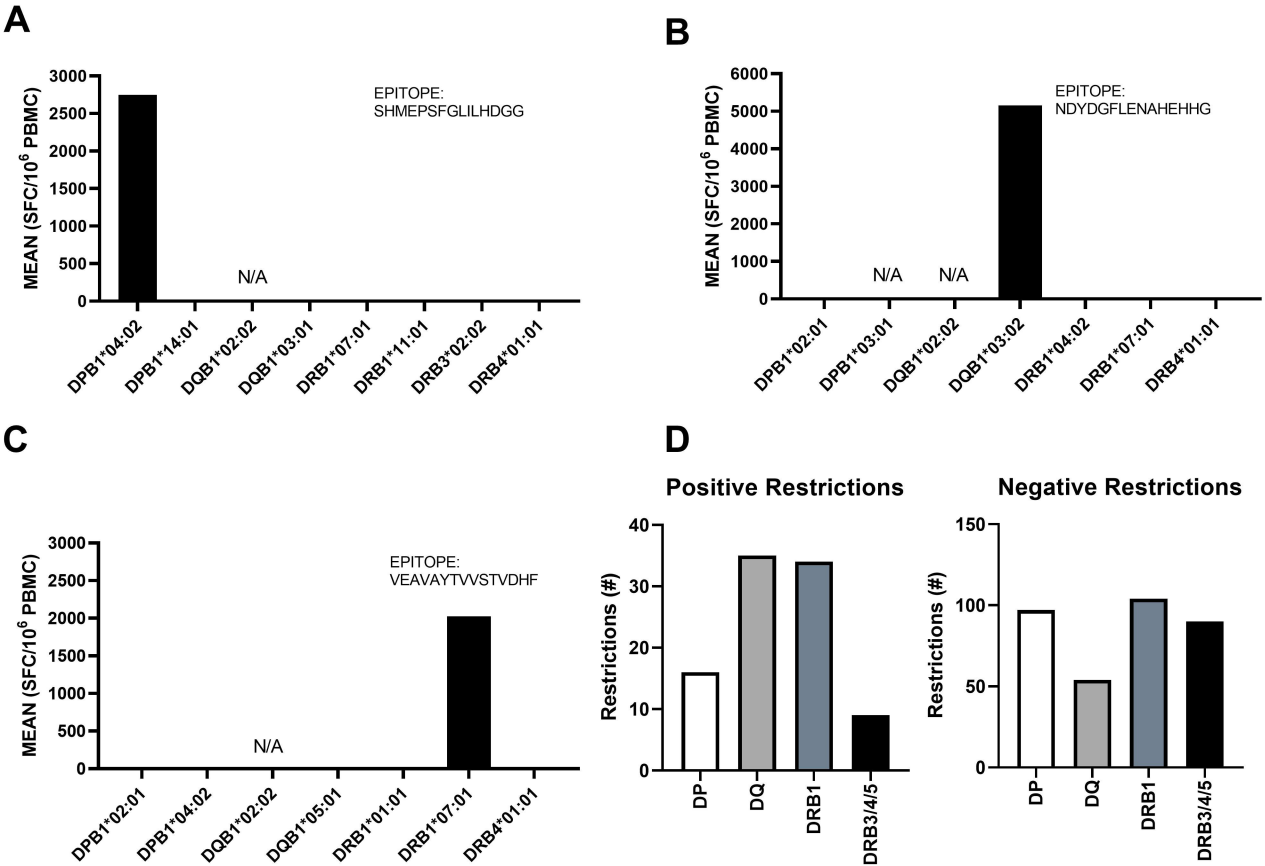

A
Cohort
General Population
Phenotype Frequency (%)
DRB1*07:01
DRB1*15:01
DRB1*03:01
DRB4*01:01
DRB3*01:01
DRB3*02:02
DRB5*01:01
DQB1*02:02
DQB1*03:01
DQB1*06:02
DQB1*03:03
DQB1*05:01
DQB1*02:01
DQB1*03:02
DQB1*05:03
DQB1*06:03
DPB1*02:01
DPB1*04:01
DPB1*04:02
DPB1*03:01
B
Representative Donor
Total SFC/10^6 PBMC (IFNγ/TNFα/IL-5)
gE P1
gE P2
gE P3
gE P4
gE P5
gE P6
gE P7
gE P8
gE P9
gE P10
gE P11
gE P12
FSVGDTFSLAMHLQY
TFSLAMHLQYKIHEA
MHLQYKIHEAPFDLL
KIHEAPFDLLLEWLY
PFDLLLEWLYVPIDP
LEWLYVPIDPTCQPM
VPIDPTCQPMRLYST
TCQPMRLYSTCLYHP
RLYSTCLYHPNAPQC
CLYHPNAPQCLSHMN
C
Total magnitude of response
Number of positive subject
Total SFC/10^6 PBMC (IFNγ/TNFα/IL-5)
# of subject responding

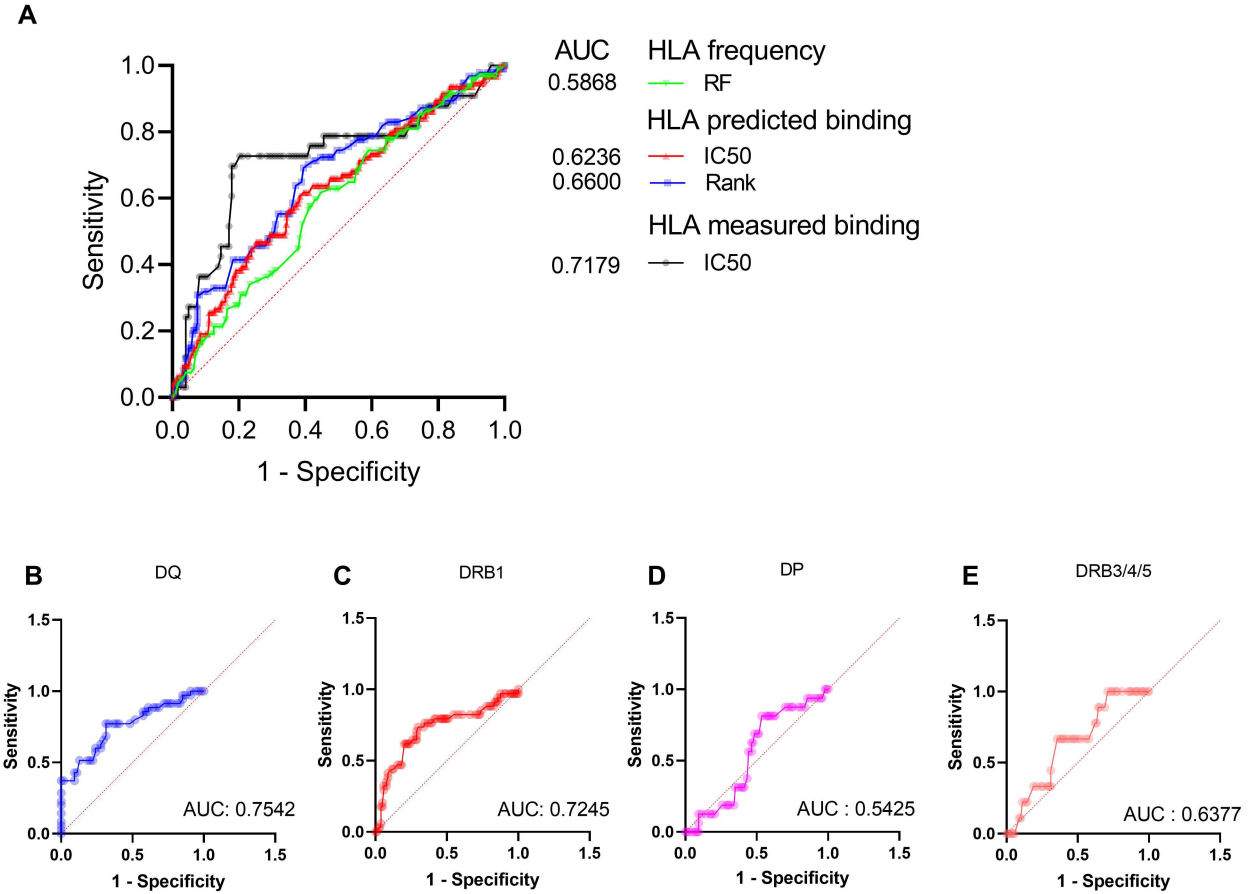

A
# of epitopes recognized
40
35
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
# of donors
B
% of total magnitude
100
90
80
70
60
50
40
30
20
10
0
0 20 40 60 80 100
# of epitopes

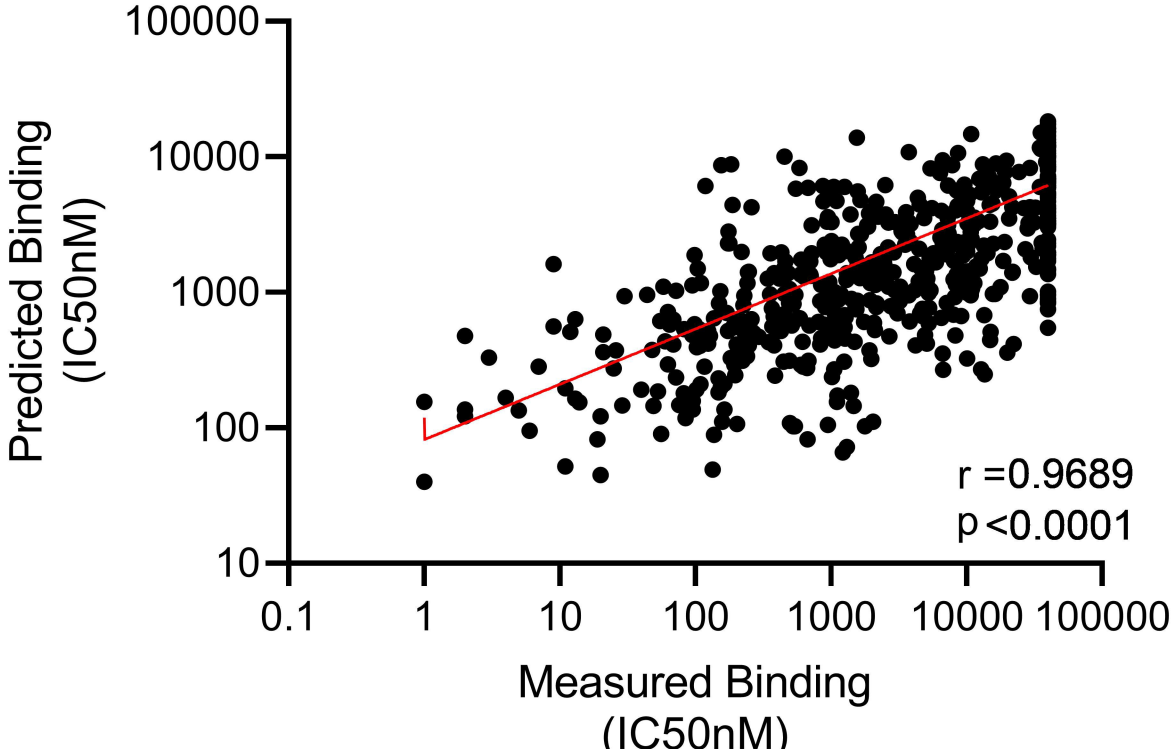

A
****
150
100
50
0
# of epitopes recognized
Recognized
Not Recognized
Shingrix
Zostavax/
Childhood-
Exposure
Both

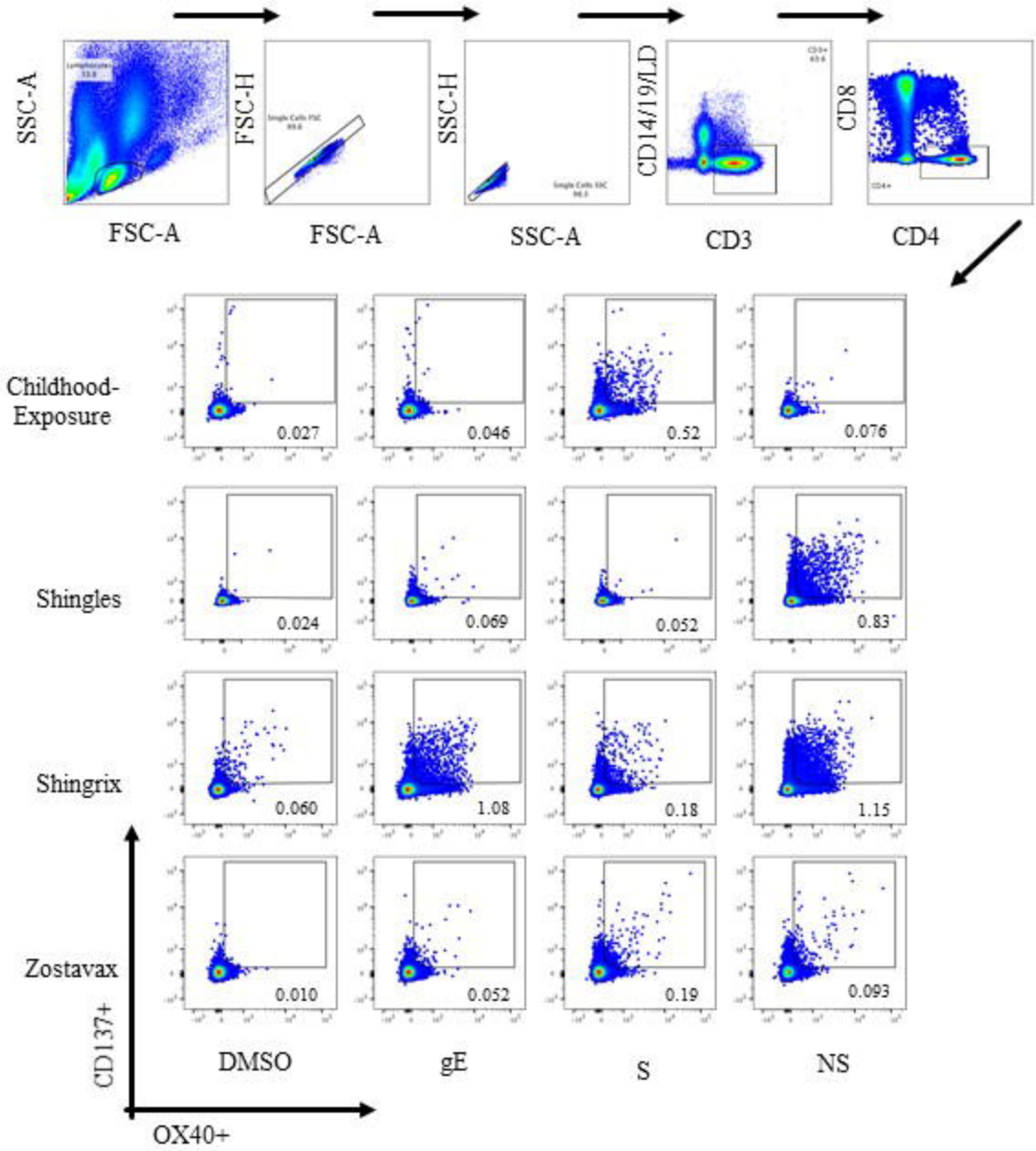

B
ns
50
40
30
20
10
0
# of epitopes recognized
Shingrix
Zostavax/
Childhood-
Exposure


C
ns
300000
200000
100000
0
SFC/ 10^6 PBMC
Shingrix
Zostavax/
Childhood-
Exposure


A
MEAN (SFC/10^6 PBMC)
3000
2500
2000
1500
1000
500
0
EPITOPE:
SHMEPSFGLILHDGG
N/A
DPB1*04:02
DPB1*14:01
DQB1*02:02
DQB1*03:01
DRB1*07:01
DRB1*11:01
DRB3*02:02
DRB4*01:01
B
MEAN (SFC/10^6 PBMC)
6000
5000
4000
3000
2000
1000
0
EPITOPE:
NDYDGFLENAHEHHG
N/A
N/A
DPB1*02:01
DPB1*03:01
DQB1*02:02
DQB1*03:02
DRB1*04:02
DRB1*07:01
DRB4*01:01
C
MEAN (SFC/10^6 PBMC)
3000
2500
2000
1500
1000
500
0
EPITOPE:
VEAVAYTVVSTVDHF
N/A
DPB1*02:01
DPB1*04:02
DQB1*02:02
DQB1*05:01
DRB1*01:01
DRB1*07:01
DRB4*01:01
D
Positive Restrictions
Restrictions (#)
40
30
20
10
0
DP
DQ
DRB1
DRB3/4/5
Negative Restrictions
Restrictions (#)
150
100
50
0
DP
DQ
DRB1
DRB3/4/5


A
AUC
HLA frequency
0.5868
RF
HLA predicted binding
0.6236
IC50
0.6600
Rank
HLA measured binding
0.7179
IC50
Sensitivity
1 - Specificity
B
DQ
AUC: 0.7542
C
DRB1
AUC: 0.7245
D
DP
AUC : 0.5425
E
DRB3/4/5
AUC : 0.6377


100000
10000
1000
100
10
Predicted Binding
(IC50nM)
0.1
1
10
100
1000
10000
100000
Measured Binding
(IC50nM)
r =0.9689
p <0.0001


SSC-A
FSC-A
FSC-H
FSC-A
SSC-H
SSC-A
CD14/19/LD
CD3
CD8
CD4
Childhood-Exposure
0.027
0.046
0.52
0.076
Shingles
0.024
0.069
0.052
0.83
Shingrix
0.060
1.08
0.18
1.15
Zostavax
0.010
0.052
0.19
0.093
DMSO
gE
S
NS
CD137+
OX40+