Transience of vaccine-induced HIV-1-specific CTL and definition of vaccine “response”

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Abstract

Many vaccine approaches emphasize producing HIV-1-specific CD8+ T-lymphocyte (CTL) responses. Towards this goal, many studies simply classify vaccinees as “responders” or “nonresponders,” based on arbitrary cutoff criteria. HIV-1-uninfected participants receiving the TBC-3B vaccine were assessed for HIV-1-specific CTL by interferon-γ/H9253 ELISpot, and compared to HIV-1-infected control subjects not on antiretroviral therapy. Vaccinees also were tested for HIV-1-specific antibody responses and generalized CD8+ T-lymphocyte activation. Different criteria for vaccine “responder” status were applied to the measured CTL values. The vaccinees showed evidence of vaccine exposure by CD8+ T-lymphocyte activation and HIV-1-specific antibodies. Considering any single positive HIV-1-specific CTL measurement a vaccine “response,” all vaccinees could be classified as “responders,” but even slight increases in the stringency of response criteria resulted in a steep decline of the “response” rate. In contrast, HIV-1-infected persons were clearly “responders” against the same proteins by the same criteria. Quantitative assessment of CTL demonstrated low and transient HIV-1-specific CTL compared to natural infection. These analyses emphasize the pitfalls of summarizing vaccine study results using simple cutoff criteria to define response rates, and suggest the utility of more comprehensive descriptions to describe vaccine immunogenicity and persistence of responses.

Keywords: HIV-1; Vaccine; CTL

1. Introduction

Clinical correlative and experimental data have indicated that HIV-1-specific CD8+ T lymphocytes (CTL) play an important protective role in HIV-1 pathogenesis, and this has prompted a vaccine development focus on generating such responses (reviewed in [1,2]). The requirement of CTL for antigen presentation through the major histocompatibility class I (MHC-I) pathway has presented an obstacle to trying to achieve this goal because this pathway generally requires endogenous expression of the targeted proteins. While a live-attenuated virus would be expected to provoke an HIV-1-specific CTL response, safety concerns have precluded developing this approach, which has been successful for other viral diseases. Attempted alternative approaches have included other methods to provide cellular expression of HIV-1 proteins, such as DNA vaccination and recombinant viral vectors containing HIV-1 genes.

Natural infection provokes vigorous HIV-1-specific CTL responses in most infected persons, and the SIV-macaque model clearly demonstrates that CD8+ T-lymphocyte responses are crucial for viral control [3–5]. Although HIV-1-specific CTL in good versus poor immunologic controllers of
HIV-1 infection cannot clearly be distinguished by commonly utilized CTL assays [6,7], a vaccine that produces CTL responses similar to natural infection has been considered an important starting goal for vaccinologists. Many vaccine trials have turned to assays such as the IFN-γ ELISpot for measurement of CTL induction due to the sensitivity of the assay, the ability to provide comprehensive assessment of epitope recognition, and the extensive data available from such measurements of HIV-1-infected individuals [8,9]. Vaccine trials often define vaccinees as “responders” or “nonresponders” based on cutoff criteria within these assays. By this type of analysis, several vaccines have shown promising results, with “response” rates approaching 90% of vaccinated individuals.

Here we examine how simply classifying vaccinees as “responders” based on cutoff criteria may be an incomplete assessment of vaccine effects. HIV-1-uninfected participants in a recombinant vaccinia virus trial are assessed and compared to HIV-1-infected persons for HIV-1-specific CTL responses using the commonly utilized methodology of IFN-γ ELISpot.

2. Methods and materials

2.1. HIV-1 uninfected vaccinees and HIV-1-infected research participants

For vaccination, six participants were enrolled on the basis of being vaccinia-naive and HIV-1-seronegative, without a history of frequent receptive anal intercourse and at low risk of being vaccinia-naive and HIV-1-seronegative, without a history of frequent receptive anal intercourse, and at low risk of being vaccinia-naive and HIV-1-seronegative, without a history of frequent receptive anal intercourse, and at low risk of being vaccinia-naive and HIV-1-seronegative, without a history of frequent receptive anal intercourse.

For vaccination, six participants were enrolled on the basis of being vaccinia-naive and HIV-1-seronegative, without a history of frequent receptive anal intercourse, and at low risk of being vaccinia-naive and HIV-1-seronegative, without a history of frequent receptive anal intercourse. For this study, we examined them as a single group without distinguishing the vaccination arm.

2.2. Vaccine

The vaccine used for these studies was Therion Recombinant Vaccinia-HIV-1 IIIB env/gag/pol vaccine (TBC-3B, Therion Biologics Corporation, Cambridge, MA, USA), a live vaccinia virus containing env, gag, and pol sequences derived from HIV-1IIIB.

2.3. Vaccination protocol

The vaccine research participants were immunized in the inguinal or deltoid areas as per a previously described targeted iliac lymph node (TILN) protocol [10]. A dose of 10⁶ PFU was administered at Week 0, followed by 10⁶ PFU immunizations in Weeks 6 and 20, via 1 ml injections subcutaneously (s.c.) in the deltoid or inguinal regions. For inguinal injections, the vaccine was administered medial to the femoral vein to optimize delivery to the inguinal and external iliac lymph nodes. Blood and sigmoid mucosal biopsies were taken at two baseline visits (2 weeks before and then immediately pre-vaccination: the mean values are presented as visit MV02), then 2 weeks after each vaccination (visits MV07, MV12, and MV18), and 32 weeks after the first vaccination (MV20). These six participants were part of a larger double-blinded study on mucosal immunity; for this study we examined them as a single group without distinguishing the vaccination route.

2.4. Evaluation of HIV-1-specific serum antibodies

Standard serum HIV-1 ELISA was performed by the UCLA Medical Center clinical laboratories.

2.5. Evaluation of HIV-1-specific CD8+ T-lymphocyte responses

CTL responses were assessed by standard IFN-γ ELISpot assays as previously reported [11,12]. Briefly, polyclonally expanded peripheral blood CD8+ T lymphocytes were screened by IFN-γ ELISpot against a library of HIV-1 peptides (consecutive 15mers overlapping by 11 amino acids) spanning all HIV-1 proteins, obtained from the NIH AIDS Research and Reference Reagent Repository (Gag catalog #8116, Pol #6208, Env #9487, Nef #5189, Tat #5138, Rev #6445, Vpr #6447, Vps #6444, Vis #6446, all Clade B consensus sequences with the exception of Env). Peptides were screened in 53 pools of 12–16 peptides each, and added to the wells at a final concentration of 5 μg/ml each. Each plate also included three negative control wells with cells but no peptides, and a positive control well containing 0.4 μg/ml of anti-CD2/CD2R and anti-CD28 monoclonal antibodies. The assays were quantitated using an automated ELISpot count-

<table>
<thead>
<tr>
<th>Participant</th>
<th>HIV-1 infection</th>
<th>Age</th>
<th>CD4</th>
<th>Viremia</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>−</td>
<td>32</td>
<td>787</td>
<td>UD</td>
</tr>
<tr>
<td>B</td>
<td>−</td>
<td>30</td>
<td>848</td>
<td>UD</td>
</tr>
<tr>
<td>C</td>
<td>−</td>
<td>34</td>
<td>1364</td>
<td>UD</td>
</tr>
<tr>
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<td>−</td>
<td>34</td>
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<td>UD</td>
</tr>
<tr>
<td>E</td>
<td>−</td>
<td>25</td>
<td>1139</td>
<td>UD</td>
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<td>F</td>
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<td>30</td>
<td>768</td>
<td>UD</td>
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<td>1</td>
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<td>2</td>
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<td>54</td>
<td>484</td>
<td>UD</td>
</tr>
<tr>
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<td>+</td>
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<td>7</td>
<td>+</td>
<td>46</td>
<td>245</td>
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<td>8</td>
<td>+</td>
<td>45</td>
<td>423</td>
<td>16,800</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>54</td>
<td>222</td>
<td>338,202</td>
</tr>
</tbody>
</table>

For the HIV-1-uninfected vaccinees (participants A–F) and HIV-1-infected control subjects (participants 1–9), the age, peripheral blood CD4+ T lymphocyte absolute count (per mm³), and HIV-1 RNA genomes per ml blood (UD ≤ 200) at the time of study are given.
Table 2  
Vaccine humoral responses against HIV-1

<table>
<thead>
<tr>
<th>Participant</th>
<th>Baseline (Week 0)</th>
<th>MV07 (Week 2)</th>
<th>MV12 (Week 8)</th>
<th>MV18 (Week 22)</th>
<th>MV20 (Week 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>−±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B</td>
<td>−−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C</td>
<td>−−</td>
<td>−</td>
<td>+</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Results of HIV-1 ELISA testing are shown for the six vaccinees receiving TCB-3B. Visits MV07, MV12, and MV18 are 2 weeks post-vaccination visits (for vaccinations given at Weeks 0, 6, and 20, respectively), and MV20 is a late follow-up 26 weeks after the third vaccination.

3. Results

3.1. TBC-3B vaccination induced humoral responses against HIV-1 and CD8+ T-lymphocyte activation

Six vaccinia-naive and HIV-1-seronegative participants at low risk for HIV-1 infection (subjects A–F, Table 1) received subcutaneous vaccinations with the TBC-3B vaccine, a recombinant vaccinia virus expressing HIV-1 Gag/Pol/Env proteins, at Weeks 0, 6, and 20. All vaccinees demonstrated detectable HIV-1-specific antibodies in the plasma by 8 weeks after the initial vaccination (Table 2), and consistently detectable IFN-γ/H9253 ELISpot responses against vaccinia virus (data not shown). In addition, flow cytometric analysis of peripheral blood CD8+ T lymphocytes demonstrated a clear trend of CD8+ T-lymphocyte activation after the first vaccination (Fig. 1), suggesting generalized cellular immune stimulation by the vaccine. These data indicated that the TBC-3B vaccine was successful both in stimulating an immune response and expressing potentially immunogenic HIV-1 proteins in vivo after vaccination.

3.2. Using cutoff criteria to define vaccine “responders” yields widely varying response rates depending on stringency of the criteria

HIV-1-specific CD8+ T lymphocyte (CTL) responses were assessed twice pre-vaccination, again 2 weeks after each vaccination, and 6 months after the last vaccination. These responses were measured by standard IFN-γ ELISpot assays using 53 pools of overlapping peptides (15mers overlapping by 11 amino acids) spanning the entire HIV-1 proteome. 38/53 peptide pools represented Gag, Pol, and Env (proteins in the TBC-3B vaccine) and 15/53 peptide pools represented Tat, Rev, Vpr, Vpu, Vif, and Nef (proteins not in the vaccine). To analyze these vaccinee CTL responses in a manner analogous to other HIV-1 vaccine trials, ELISpot measurements against each peptide pool were scored as positive or negative based on fixed cutoff criteria (Fig. 2 and supplemental Table 1). CTL recognition of peptide pools was defined as at least 50 SFC above background per million CD8+ T lymphocytes and at least twice the background value (mean for no-peptide controls). The vaccinees were then categorized as vaccine “responders” or “nonresponders” based on their numbers of recognized peptide pools, using varying criteria (Fig. 3). For comparison, nine chronically HIV-1-infected persons were analyzed in parallel.

All six vaccinees (100%) appeared to be “responders” as defined by having any single detected single CTL recognition of any HIV-1 peptide pool occurring at any time after vaccination. However, even slight increases in the stringency of the criteria for vaccine “response” resulted in a rapid drop in “responders”; when criteria were increased to consider persons with more than a single positive CTL measurement, two of the vaccinees (33%) had at least three detectable CTL responses post-vaccination (Figs. 2 and 3). If reproducibility of CTL recognition was included as the criteria, the reduction in participants considered “responders” was even more dramatic. Only two vaccinees (33%) had any single CTL peptide pool response that was repeatable on more than one visit, and none had even two different CTL responses that were repeatable on more than one visit. By comparison, chronically HIV-
Fig. 2. CD8+ T-lymphocyte responses of vaccinees and HIV-1-infected persons against HIV-1 peptide pools. CD8+ T lymphocytes from the TBC-3B vaccinees and HIV-1-infected individuals were analyzed for responses against HIV-1 peptide pools (38 pools spanning Gag, Pol, and Env) by IFN-γ ELISpot assay. Recognition of pools was defined as at least 50 spot-forming cells per million cells and exceeding twice the background value for the no-peptide controls (see supplemental table). The six vaccinees were evaluated over four post-vaccination assays (2 weeks after each of the three vaccinations at Weeks 0, 6, and 20, and 6 months after the third vaccination), and the nine HIV-1-infected participants were evaluated in two assays that were 2 weeks apart. Responses vaccine proteins (peptide pools for Gag, Pol, or Env) are plotted for the number of peptide pools recognized on any single visit (A) or the number of peptide pools recognized on at least two different visits (B).

Fig. 3. Classification of vaccinee and HIV-1-infected participant “response” rate to HIV-1 Gag, Pol, and Env using differing cutoff criteria. For each of the different cutoff criteria presented in Fig. 2, the CTL “response” rate to the Gag, Pol, and Env proteins is plotted for the HIV-1-uninfected vaccinees and the HIV-1-infected control participants: (A) “response” classified by the number of peptide pools (Gag/Pol/Env) recognized at any given visit and (B) “response” classified by the number of pools (Gag/Pol/Env) recognized on more than a single visit. Shaded bars represent vaccinees, black bars HIV-1-infected participants.

1-infected persons demonstrated much higher “responder” rates using the same assays and criteria, even though analyzed for only two visits rather than the four post-vaccination visits of the vaccinees (Figs. 2 and 3). Nine of nine infected individuals (100%) were “responders” when defined as having at least four CTL responses that were observed at both visits. Overall, these data demonstrate that the vaccine “response” rates can vary dramatically depending on small adjustments to the defining criteria, in contrast to naturally infected persons. Simply using total positive wells as the criteria to define vaccine response yields varying results and fails to reflect the prevalence of reproducible of CTL responses.

3.3. TBC-3B vaccination induced weak and transient global CD8+ T-lymphocyte responses against HIV-1 compared to natural infection

To assess the global CTL responsiveness to vaccination, we compared the mean CTL response magnitude to the peptide pools representing HIV-1 proteins contained within the vaccine (Gag/Pol/Env) to the mean magnitude against peptide pools for proteins not in the vaccine (Tat/Rev/Vpr/Vpu/Vif/Nef). This approach thus utilized the detected responses against non-vaccine proteins as a control for non-specific background detection in this system. The
A mean of 57 CTL responses against the same Gag/Pol/Env peptide pools in participants not on antiretroviral treatment for their mean pretreatment baseline measurements demonstrated little difference between these parameters (Fig. 4A), indicating a general lack of vaccine-specific CTL activity above background at baseline. In three individuals, there was a trend for a 64% vaccine response rate among volunteers vaccinated with TCB-3B vaccine, the value for the mean ELISpot responses against vaccine HIV-1 protein (Gag, Pol, Env) minus the mean responses against non-vaccine HIV-1 proteins (Nef, Rev, Tat, Vpr, Vpu, Vif) are plotted, the arrows indicate vaccinations and (B) for the nine HIV-1-infected participants receiving the TBC-3B vaccine, the value for the mean background-subtracted ELISpot response against Gag/Pol/Env is plotted.

Fig. 4. CTL responses against vaccine HIV-1 proteins in uninfected vaccinees and HIV-1-infected participants (A) for each of six HIV-1-uninfected participants receiving the TBC-3B vaccine, the value for the mean ELISpot responses against vaccine HIV-1 protein (Gag, Pol, Env) minus the mean responses against non-vaccine HIV-1 proteins (Nef, Rev, Tat, Vpr, Vpu, Vif) are plotted; the arrows indicate vaccinations and (B) for the nine HIV-1-infected control subjects, the mean background-subtracted ELISpot response against Gag/Pol/Env is plotted.

4. Discussion

Although the precise correlates of protective immunity against HIV-1 infection remain obscure, it is clear that CTL responses similar in magnitude to that of natural HIV-1 infection are critical to the control of HIV-1 replication and most likely play a role in protective immunity [1,13]. For this reason, the majority of vaccines currently being tested include immunogens to induce CTL responses [14-16]. To this end, various strategies such as recombinant viral vectors and DNA vaccination are currently being tested in FDA phase I and II trials. However, due to the low incidence rate for HIV-1 infection in many areas where these trials are being conducted, laboratory endpoints tend to be used for vaccine evaluation.

Recently developed techniques such as the IFN-γ ELISpot exhibit remarkable technical simplicity and sensitivity for detecting HIV-1-specific CTL [8,9]. However, the use of such data to provide a “correlate of immunity” remains elusive, with no clear understanding of how CTL measurements might correlate to protection against infection, modulation of the rate of progression to AIDS, or reduced transmission [1,15]. Even in chronically HIV-1-infected persons with obviously different levels of immune control, it has been difficult to find any pattern or quantity of CTL measurements that correlate with control. This clearly poses challenges for evaluating the capability of a vaccine to elicit protective immunity in vaccinees. Given this gap in knowledge, vaccine trials often have focused on achieving immunogenicity generating CTL responses similar in magnitude to that of natural HIV-1 infection, as a first step.

Vaccination with TCB-3B demonstrates a “response rate” that varies greatly with the stringency of the applied criterion for defining “responders.” Using the least stringent criterion of any single positive CTL measurement over time, this study revealed a promising response rate of 6/6 or 100%. This result is similar to that of several preventive vaccine candidates reporting promising results in clinical trials, using similar cumulative cutoff criteria. For example, phase I trials of DNA and recombinant modified Vaccinia Virus Ankara (MVA) HIV-1 vaccines have reported response rates between 78 and 89% depending on the vaccine strategy employed [17]. Most of these studies, however, have not revealed the reproducibility of vaccine CTL responses over time. Our data indicate that when the persistence of HIV-1-specific CTL over time is considered within the definition of CTL “response,” there are few vaccine “responders.” This finding agrees with other discouraging vaccine studies reporting on the reproducibility of CTL detection. For example, Belshe et al. found a 64% vaccine response rate among volunteers vaccinated with ALVAC-HIV (vCP205) when defined as having any single detected CTL response by chromium release assay against Gag or Env (responses against whole proteins, as opposed to our ELISpot using peptide pools corresponding to small regions of proteins), while only 25% of these vaccinees had more than a single positive measurement over time [18].

Is reproducibility of responses an important component for defining a “responder” to vaccination, when attempting to predict vaccine efficacy? One consideration is the accuracy of detection; for vaccines where CTL responses are near the lower limits for detection, reproducible responses over time are less likely to be false positives. Even if robust HIV-1-specific CTL responses comparable to natural infection can be elicited by a vaccine, another consideration is the possibility that sustained responses are important for protection. Kaul et al. found that up to 50% of uninfected commercial sex workers had detectable HIV-1-specific CTL responses after 3 years of ongoing sexual exposures, but that sex workers taking a hiatus showed waning responses and were subsequently at increased risk for infection after renewed exposures [19,20]. These results suggest that durability of responses should be an important goal for HIV-1 preventive vaccines.

Despite good immunologic control of an initial HIV-1 strain, infected persons can become superinfected by HIV-1 strains of the same clade [21,22]. This indicates that vaccine...
induced HIV-1-specific CTL with a magnitude and persistence similar to natural infection may be necessary but not sufficient to prevent infection. It is striking in the current study that vaccination with recombinant TBC-3B yields a "response rate" ranging from promising to dismal depending on the criteria utilized, in the setting of provoking CTL levels far lower than those in natural infection. Thus, while the "correlate of immunity" from such measurements as ELISpot remains unknown, it seems likely that classifying vaccinees as "responders" is overly simplistic. Absent a clear understanding of protective immunity, multiple parameters such as the magnitude, breadth, and durability of HIV-1-specific CTL responses should be examined and reported in vaccine trials.

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References