Vaccine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Comparison of the patterns of antibody recall responses to HIV-1 gp120 and hepatitis B surface antigen in immunized mice

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ARTICLE INFO

Article history:
Received 22 January 2016
Received in revised form 10 August 2016
Accepted 24 October 2016
Available online xxxx

Keywords:
HIV-1
Envelope
HBsAg
T follicular helper cells
PD-1
Immune memory

ABSTRACT

To date, we still lack an ideal strategy for designing envelope glycoprotein (Env) vaccines to elicit potent protective antibodies against HIV-1 infection. Since the human hepatitis B virus surface antigen (HBsAg) is representative of effective vaccines that can induce ideal humoral immune responses, knowledge of how it elicits antibody responses and T helper cells would be an useful reference for HIV vaccine development. We compared the characteristics of the HIV-1 Env gp120 trimer and HBsAg in antibody elicitation and induction of T follicular helper (Tfh) and memory B cells in immunized Balb/c mice. Using the strategy of protein prime-protein boost, we found that HIV-1 gp120 induced slower recall antibody responses but redundant non-specific IgG responses at early time after boosting compared to HBsAg. The higher frequency of PD-1^{hi}CD4^{+} T cells and Tfh cells that appeared at the early time point after gp120 boosting is likely to limit the development of memory B cells, memory T cells, and specific antibody recall responses. These findings regarding the different features of HIV envelope and HBsAg in T helper cell responses may provide a direction to improve HIV envelope immunogenicity.

1. Introduction

Systematic analyses of HIV-1 positive sera have revealed that about 5–25% of HIV-1 infected individuals develop broadly neutralizing antibody (bNAb) responses, and approximately 1% of individuals are classified as elite neutralizers [1–4]. Although a number of monoclonal bNAbs against the HIV-1 envelope (Env) have been discovered [5–7] and the epitopes of such bNAbs have been well elucidated, we still face a major challenge in eliciting similar bNAbs by immunization [8]. Env, especially gp120, is the primary target for HIV-1 vaccine design. To date, only few Env constructs including a native-structure-mimic Env, gp140 SOSIP [9], and the gp120 outer domain with delimited glycans, OD_{EC} [10], have been shown to elicit potent NAb responses in immunized animals. However, a majority of Env protein immunogens could not elicit potent NAb responses [11]. Therefore, we still lack an ideal strategy for designing Env vaccines to elicit protective antibodies against HIV-1 infection.

The viral surface antigen (HBsAg) has been used worldwide for more than 30 years as a vaccine against hepatitis B virus (HBV) infection. It is the most effective vaccine in reducing the global incidence of hepatitis B [12]. More than 90% of immunocompetent adults produce protective antibody responses after HBsAg vaccination [13]. Indeed, rapid protection can be achieved after three doses of vaccine administration. Successfully vaccinated individuals usually show a rapid recall response to a booster after several years since the primary vaccination or upon exposure to HBV [14]. This indicates that immunization with HBsAg may elicit an ideal immune memory and provide long-term protection [15]. In addition, some viral antigens such as human papillomavirus (HPV) L1 protein [16,17] and the influenza virus hemagglutinin protein (HA) also elicit considerable antibody levels [18,19] providing protection against viral infection.

It has been reported that HBsAg-induced protection is mediated by two mechanisms: immediate neutralization of HBV by anti-HBs antibodies and activation of specific CD4^{+} T memory cells that promote subsequent activation of memory B cells and secretion of

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http://dx.doi.org/10.1016/j.vaccine.2016.10.063
0264-410X/

anti-HBs antibodies [13]. HBsAg-specific memory T and B cells still exist in vaccinated individuals even when the specific antibody levels decline [20].

T follicular helper cells (Tfh) have been identified as an important T cell subset involved in T and B cell interaction, antigen-specific B cell differentiation, and development of high-affinity memory B cells and antibody-producing cells [21]. Tfh cells are required for affinity-maturation of B cells in germinal centers (GCs). Because of the key roles of Tfh in producing protective antibodies against T-cell-dependent antigens, it is essential to explore the events that affect Tfh differentiation and the interaction of T and B cells in the context of vaccination using various kinds of immunogens.

Apparently, many efforts have been made to elucidate the mechanisms of less potent neutralizing antibody responses induced by HIV Env immunization. However, not many side-by-side comparisons of HIV-1 Env with other antigens are available. Sundling et al. have found that immunization of macaques with soluble HIV-1 Env and influenza virus hemagglutinin (HA) resulted in rapid contraction of peripheral B-cell responses after boosting. They claimed that short-lived memory responses might not be unique to HIV-1 Env but may be a common feature of soluble protein vaccines [22]. Daly et al. have found that HIV gp120 may induce more Th2-biased responses compared with HA [23]. Anderson et al. have reported that the HIV gp120 protein induced slower humoral immune responses and rapid decline of antibody titers in baboons, compared to that by HBsAg [24]. Further studies to explore the mechanisms underlying these different patterns have not been reported. Since HBsAg is representative of effective vaccines that can induce ideal humoral immune responses, elucidating the possible reasons for the different patterns of specific humoral immune responses by HIV Env and HBsAg may be helpful for HIV vaccine development.

Here, we compared the patterns of antibody responses and the induction of Tfh and memory B cells induced by the HIV-1 Env gp120 trimer and HBsAg in immunized mice. We found that gp120 induced slower recall antibody responses but redundant non-specific antibody responses at early time after boosting, compared to HBsAg. The phenomena observed in gp120 immunization were associated with induction of PD-1^{hi} T cells after boosting. The present findings may thus provide a direction to improve HIV envelope immunogenicity.

2. Materials and methods

2.1. Antigens and adjuvant

The HIV-1 06044 gp120 trimer protein was prepared as described previously [25]. HBsAg was purchased from GENIA Biotechnology Company (Beijing, China). Both proteins were identified by native-PAGE and western blotting. Rabbit anti-gp120 antibody (Sino Biological, Beijing, China) and goat anti-HBsAg (Bioss, Beijing, China) were used for western blotting. Horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG (ZSGB-BIO, Beijing, China) and HRP-labeled rabbit anti-goat IgG (ZSGB-BIO) were used as secondary antibodies. The proteins were formulated with an equal volume of the adjuvant AddaVax (InvivoGen, San Diego, California, USA) and were then used as immunogens.

2.2. Immunization and specimen harvest

Female Balb/c mice (8-week old) were purchased from Vital River Laboratories and housed in the Animal Laboratory of Harbin Medical University. Mice were immunized subcutaneously with adjuvant-formulated gp120 protein (8 μg per mouse) or HBsAg (2 μg per mouse) at weeks 0, 3, 6, 9, and 12. The immunization dose for gp120 protein was determined based on our previous work wherein we administered 2 μg of gp120 protein per mouse for immunization and found that this dose of the immunogen did not induce a sufficient humoral immune response. Instead of the specific immunogen, AddaVax alone in PBS was used as the negative control. At days 3, 7, and 14 after the final immunization, the sera, splenocytes, and bone marrow cells were harvested from the mice (Fig. 1c). All the mice used in the experiments were treated according to the guidelines of the Animal Care Committee of Harbin Medical University (HMUIRB20140020).

2.3. Detection of serum IgG and specific antibodies

Serum IgG levels were quantified using the Mouse IgG total ELISA Ready-SET-Go Kit (eBioscience, San Diego, California, USA) following the manufacturer's instructions and as described previously [25].

HIV-1 Env-specific antibodies in the serum samples were analyzed using ELISA as described previously [25]. To detect the Ag-specific antibodies, 96-well plates (JET BIOFIL, Guangzhou, China) were coated at 4 °C overnight with HIV-1 Bal gp120, JRCSF gp120 (Both proteins were obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH), 06044 gp120 trimer protein [26], or HBsAg at a final concentration of 0.5 μg/mL. After blocking with PBS containing 0.5% Tween-20 and 1% BSA, 10-fold serially diluted HBsAg-immunized sera or 2-fold/5-fold serially diluted gp120-immunized sera were added and incubated at 37 °C for 2 h. The plates were then washed and incubated with HRP-labeled goat anti-mouse IgG (ZSGB-BIO) at a dilution of 1:1000 for 30 min at room temperature (RT). After washing, the substrates were added and the reactions were stopped with 2M H_2SO_4. The absorbance at 450 nm (OD450) was measured using the Model 550 Microplate Reader (Bio-Rad, Hercules, California, USA).

2.4. B cell ELISpot assay

The B cell ELISpot assay was performed using the ELISpot^{PLUS} for Mouse IgG kit (Mabtech, Nacka Strand, Sweden) as described previously [25]. Briefly, 96-well MultiScreen-IP filter plates were coated with 10 μg/mL of polyclonal goat anti-mouse IgG. After blocking, appropriate numbers of bone marrow cells (1×10^6 for specific IgG- or 1×10^5 for total IgG-secreting cell detection) were added per well and incubated at 37 °C for 16–24 h. Then, 100 μL/well of biotin-labeled gp120, HBsAg, or biotin-anti-mouse IgG was added and incubated at room temperature for 2 h. Incubation with alkaline phosphatase (ALP)-conjugated streptavidin, the substrate reaction, and the stop solution, were performed as the manufacturer's instructions. Spots representing the specific antibody-secreting cells (ASCs) and the total IgG ASCs were counted using the ChampSpot III ELISpot Analysis System (Beijing, China).

2.5. Serum immunoglobulin subclass analysis by ELISA

An ELISA assay was performed to measure the endpoint titers of Ag-specific immunoglobulin (Ig) subclasses in mouse serum samples. Flat bottomed96-wellplates were coated at 4 °C overnight with JRCSF gp120 (NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH) or HBsAg (GENIA Biotechnology Company, Beijing, China) at a final concentration of 0.5 μg/mL in coating buffer (0.05 mol/L Na_2CO_3, 0.05 mol/L $NaHCO_3$, pH9.6). After blocking with PBS containing 0.5% Tween-20 and 1% BSA, 10-fold serially diluted HBsAg-immunized sera or 2-fold serially diluted gp120-immunized sera were added to each well (100 μL/well) and incubated at 37 °C for 2 h. The plates were then washed and incubated with 1:10,000 diluted HRP-labeled antibodies against mouse IgG1,

Please cite this article in press as: Yu H-T et al. Comparison of the patterns of antibody recall responses to HIV-1 gp120 and hepatitis B surface antigen in immunized mice. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.10.063