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Research paper

Optimization and qualification of a memory B-cell ELISpot for the detection of vaccine-induced memory responses in HIV vaccine trials

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ABSTRACT

Various aspects of the human immune system can be analyzed to determine the efficacy of a vaccine. We have developed a B-cell ELISpot to measure HIV-specific antibody-secreting B cells in the peripheral blood as a result of vaccination or natural infection. Our method includes stimulating peripheral blood mononuclear cells with interleukin-2 and a polyclonal activator, R848, to induce memory B cells to differentiate into antibody-secreting cells. Total immunoglobulin-secreting as well as antigen-specific B cells are then quantified. We have tested several HIV Env gp120 and gp140 proteins from different HIV subtypes, as well as a sensitive consensus group M Env gp140. Our findings indicate that the B-cell ELISpot provides a sensitive and specific tool to detect antigen-specific memory B-cell responses, and it is equally suited to detect antibody-secreting plasmablasts present in the circulation shortly after infection or vaccination.

1. Introduction

Antibody responses are crucial for prevention of many infections, and represent a correlate of protection for almost all effective vaccines (Plotkin, 2010). Their titers are therefore the predominant measure in most vaccine trials. Memory B cells, along with terminally differentiated plasma cells, are responsible for the long-term persistence of the humoral immunity elicited by most vaccinations and some infections (West and Calandra, 1996; Crotty et al., 2003), so the assessment memory B-cell responses may provide additional information on vaccine take and longevity of vaccine-induced protection. Immunoglobulin-G (IgG) titers and memory B-cell responses can correlate (Crotty et al., 2003), but can also be distinct (Leyendeckers et al., 1999; Amanna et al., 2007), suggesting that the measurement of memory B cells is not redundant with that of circulating antibodies. The detection of antigen-specific B cells rather than circulating antibodies can also be helpful in differentiating maternal antibodies from de novo responses induced in infants upon infection or vaccination; as well as to distinguish local IgG production from transudation of serum IgG in mucosal samples.

The generation of high-affinity antibody-secreting plasma cells and memory B cells occurs within the germinal center of lymphoid tissues through somatic hypermutation and

Abbreviations: PBMC, peripheral blood mononuclear cells; Ag, antigen; Ig, immunoglobulin; ASC, antibody-secreting cell; TMB, tetramethylbenzidine; KLH, Keyhole Limpet Hemocyanin; HBSS, Hanks balanced salt solution; PVDF, polyvinylidene fluoride; SFC, spot-forming cells.

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selective expansion. These memory B cells can persist independent of the immunizing antigen (Ag) and are capable of mounting a rapid anamnestic secondary response upon re-exposure to the antigen, during which some of the memory B cells terminally differentiate into antibody-secreting plasma cells (Crotty et al., 2004; LeBien and Tedder, 2008). Different pools of long-lived plasma cells are generated after the primary and secondary exposures, which migrate to the bone marrow from the spleen and can survive for the life of the host without expanding (Manz et al., 2002; McHeyzer-Williams and McHeyzer-Williams, 2005; Radbruch et al., 2006).

In this paper, we describe the optimization of a previously published memory B-cell ELISpot assay specific for HIV-1 surface proteins in order to determine the immune stimulating effects of HIV vaccines (Crotty et al., 2004; Bonsignori et al., 2009; Dosenovic et al., 2009). This optimization focused on the stimulation conditions that result in the most robust and consistent detection of vaccine-induced memory B-cell responses, resulting in a reliable qualified assay ready to be applied in clinical trials. This assay is equally well suited to identify ex vivo antibody-secreting cells (ASCs) circulating in the blood shortly after vaccination and resident mucosal ASCs, as well as memory B cells in the periphery and mucosal tissues.

2. Material and methods

2.1. Study participants

Samples were obtained from four HIV subtype B-infected and twenty uninfected individuals enrolled in the study "Establishing Immunologic Assays for Determining HIV-1 Prevention and Control," also referred to as Seattle Area Controls or SACs. HIV-infected subjects were chronically infected and on antiretroviral treatment. In addition, we tested samples from 19 individuals enrolled in HVTN 204 (Churchyard et al., 2011). HVTN 204 is a phase II clinical trial to test the immunogenicity of a multiclade HIV-1 DNA plasmid vaccine (subtype B Gag, Pol, and Nef; subtypes A, B, and C Env) followed by a multiclade recombinant adenovirus serotype 5 vector HIV-1 vaccine boost (subtype B Gag-Pol fusion; subtypes A, B, C Env) in HIV-1 uninfected adult participants. Samples tested were taken at baseline and one month post final vaccination.

All volunteers provided informed written consent before participating in the studies, and all studies were approved by the Institutional Review Boards of the Fred Hutchinson Cancer Research Center and other participating institutions for HVTN 204.

2.2. Sample processing

Cryopreserved peripheral blood mononuclear cells (PBMC) were used for assay development, but other cell sources can equally be used in this assay (e.g., gut mucosa mononuclear cells [GMMC] obtained through flexible sigmoidoscopy). PBMC were isolated from whole blood treated with acid citrate dextrose or sodium heparin using Leucosep tubes (Greiner Bio-One, Monroe, NC) according to the manufacturer's instructions. PBMC were counted using a Coulter counter and frozen at 15 million cells/vial in cryopreservation solution (90% fetal bovine serum [FBS] with 10% DMSO). GMMC from biopsies obtained by flexible sigmoidoscopy were isolated by two rounds of digestion with collagenase II (Sigma-Aldrich, St. Louis, MO) followed by gradient centrifugation using Histopaque (Sigma, St. Louis, MO). GMMC were counted on a Guava Counter using αCD45-FITC, αCD19-PE and 7AAD (BD Biosciences, San Jose, CA) for enumeration of live B cells, and were used after overnight rest at 37 °C.

2.3. PBMC thawing

Cryopreserved PBMC were rapidly thawed in a 37 °C water bath and then slowly added to 10 ml of warmed R10 (RPMI 1640 [GibcoBRL, Carlsbad, CA], 10% FBS [Gemini Bioproducts, West Sacramento, CA], 2 mM L-glutamine [Gibco], 100 μg/ml streptomycin sulfate [Gibco], 100 U/ml penicillin G [Gibco]) containing 20 μl Benzonase (25 U/μl; Novagen, Madison, WI). The cells were then counted using the Guava ViaCount Kit (Millipore, Bedford, MA) according to manufacturer's instructions and stimulated as described in Section 2.4.

2.4. Memory B-cell stimulation

PBMC were resuspended at 1×10^6 PBMC/ml in stimulation media and incubated at 37 °C in 5% CO_2. Stimulation media and conditions were optimized as described in Section 3.3; best results were obtained using stimulation media composed of R10, 5 ng/ml interleukin 2 (IL-2, Mabtech, Mariemont, OH) and 0.5 μg/ml imidazoquinoline resiquimod (R848, Mabtech), and stimulating cells for 5 days. Stimulated cells were washed in R10 prior to plating (see Section 2.6).

2.5. HIV Envelope proteins

We tested numerous HIV Env gp140 and gp120 proteins to identify ones that were readily available and consistently detected a response with clear, defined spots in HIV-infected subjects or vaccine recipients. We selected two HIV-1 envelope (Env) proteins for general use: an HIV-1 Subtype B gp140, BaL (GenBank accession number ABC55874, Immune Tech Corporation, New York City, NY); and a consensus group M gp140, ConS (kindly provided by Dr. Georgia Tomaras, Duke University). We also tested another HIV-1 Subtype B gp120 protein, SF162 (GenBank accession number AAT67508, Immune Tech Corporation, New York, NY); however, it produced very high background staining and was therefore used to demonstrate the effects of biotinylation in background reduction and spot definition. In addition, we used the two HIV-1 proteins in AIDSVAX B/E (Billich, 2001) for testing vaccine-specific responses in clinical trials: subtype AE gp120 A244 and subtype B gp120 MN. We used EZ-Link Micro Sulfo-NHS-Biotinylation kit (Thermo Scientific, Pittsburgh, PA) to chemically bind biotin to primary amines, such as in the side-chain of lysine residues and the N-terminus of polypeptides. Because random biotinylation by chemical linking of the side-chain of lysine that is often critically important for HIV-1 antibody binding can potentially negatively impact some important epitopes such as K169 for V2 antibody binding (Rolland et al., 2012; Liao et al., 2013), alternatively, the addition of an AVI-tag (GLNDIFEAQKIEWHE) (Kay et al., 2009) to the