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Standard-Dose Intradermal Influenza Vaccine Elicits Cellular Immune Responses Similar to Those of Intramuscular Vaccine in Men With and Those Without HIV Infection

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Abstract

Background.—Human immunodeficiency virus (HIV)–infected persons are at a higher risk of severe influenza. Although we have shown that a standard-dose intradermal influenza vaccine versus a standard-dose intramuscular influenza vaccine does not result in differences

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Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

in hemagglutination-inhibition titers in this population, a comprehensive examination of cell-mediated immune responses remains lacking.

Methods.—Serological, antigen-specific B-cell, and interleukin 2–, interferon γ–, and tumor necrosis factor α–secreting T-cell responses were assessed in 79 HIV-infected men and 79 HIV-uninfected men.

Results.—The route of vaccination did not affect the immunoglobulin A and immunoglobulin G (IgG) plasmablast or memory B-cell response, although these were severely impaired in the group with a $CD4^+$ T-cell count of <200 cells/μL. The frequencies of IgG memory B cells measured on day 28 after vaccination were highest in the HIV-uninfected group, followed by the group with a $CD4^+$ T-cell count of ≥200 cells/μL and the group with a $CD4^+$ T-cell count of <200 cells/μL. The route of vaccination did not affect the $CD4^+$ or $CD8^+$ T-cell responses measured at various times after vaccination.

Conclusions.—The route of vaccination had no effect on antibody responses, antibody avidity, T-cell responses, or B-cell responses in HIV-infected or HIV-uninfected subjects. With the serological and cellular immune responses to influenza vaccination being impaired in HIV-infected individuals with a $CD4^+$ T-cell count of <200 cells/μL, passive immunization strategies need to be explored to protect this population.

Clinical trials registration.—NCT01538940.

Keywords

Influenza; vaccination; HIV; cell-mediated; intradermal; intramuscular

Influenza virus causes an estimated 291 243–645 832 fatalities annually and continues to remain a pathogen of significant public health importance [1]. Those especially susceptible to severe illness or complications from influenza virus infection include young children, pregnant women and immunocompromised individuals like those living with human immunodeficiency virus (HIV) [2]. Although the increased mortality and morbidity seen in populations of HIV-infected people following influenza virus infection [3] appear to have decreased during the highly active antiretroviral therapy era, health complications remain at levels comparable to those among other high-risk groups for which annual influenza vaccination is recommended [4].

Immune abnormalities occur shortly after HIV infection. These include a reduction in the $CD4^+$ T-cell count, a correlate of disease progression [5]; an increase in the $CD8^+$ T-cell count until the late phase, when depletion occurs [6]; and increased levels of activation, exhaustion, and turnover of B cells, resulting in altered frequencies of B-cell subsets in the peripheral blood [7]. Immune dysfunction in HIV-infected individuals may lead to impaired responses to standard-dose intramuscular influenza vaccine [8]. Thus, a continuing need to develop more-effective vaccine strategies for HIV-infected individuals exists.

One strategy is the use of the intradermal route for vaccination. Intradermal vaccination is considered to be more immunogenic because of the abundance of professional antigen-presenting cells (APCs) and a high density of blood and lymphatic vessels in the skin [9]. Previous studies have demonstrated that antibody responses were not inferior when

comparing a reduced-dose (ie, 9-μg) intradermal influenza vaccine to a standard-dose (15-μg) intramuscular influenza vaccine in HIV-infected people [10]. Subsequently, it has been shown that standard-dose intradermal vaccine did not result in higher hemagglutination-inhibition (HI) titers than standard-dose intramuscular vaccine in HIV-infected individuals [11]. Although the immunogenicity of inactivated influenza vaccines has conventionally been assessed with serological assays, evidence suggests that serological studies based on the HI assay alone may not be the best approach to assess correlates of protection induced by vaccination [12–14].

To better understand whether the route of immunization resulted in better vaccine responses, we comprehensively analyzed B-cell– and T-cell–mediated immunity (CMI) in HIV-infected versus HIV-uninfected individuals following receipt of an intradermal or intramuscular influenza vaccine in Bangkok, Thailand.

METHODS

Study Participants and Data Collection

The study was a subset of a larger trial designed to investigate vaccine responses following receipt of intradermal or intramuscular influenza vaccine among HIV-infected Thai men who have sex with men. Written informed consent was obtained from all eligible participants. Enrollment and vaccination protocols have been described in detail elsewhere [11]. Briefly, among the 400 HIV-infected participants enrolled in the serological study, 40 persons per study arm were selected to have peripheral blood mononuclear cells (PBMCs) collected at each study visit. The first 20 participants with a $CD4^+$ T-cell count of <200 or ≥200 cells/μL to enroll in each study arm (and who agreed to participate) were enrolled in the CMI portion of the study. In addition, 80 HIV-uninfected participants also had PBMCs, as well as sera, collected at each study visit. All individuals selected to undergo CMI studies were required to provide a 30-mL venous blood specimen at each study visit for PBMC collection at baseline, 7 days, 1 month, and 6 months after vaccination, in addition to the 10 mL collected for the evaluation of serological responses. Participants in each group were randomly assigned to receive either intramuscular or intradermal vaccine. The intramuscular (Vaxigrip) and intradermal (Intanza or IDflu) vaccines were inactivated, standard-dose, split-virion, trivalent influenza vaccines (2011 northern hemisphere and 2012 southern hemisphere vaccine components: A/California/7/2009[H1N1]-like virus, A/Perth/16/2009[H3N2]-like virus, and B/Brisbane/60/2008–like virus). Randomization arms were well balanced with respect to age, $CD4^+$ T-cell count, HIV RNA load, and antiretroviral treatment status (Table 1).

Laboratory Methods

Specimen Collection and Handling—The details of influenza serological testing have been described elsewhere [11]. Thirty-milliliter whole-blood specimens were collected from study participants, using Vacutainer tubes containing ethylenediaminetetraacetic acid or heparin, and processed within 24 hours of collection. PBMCs were isolated from whole-blood specimens by Ficoll-Paque density gradient centrifugation at room temperature, counted, resuspended at 5×10^6 cells/mL in freezing medium containing 90% fetal bovine

serum and 10% dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO), and transferred to cryotubes in 1-mL aliquots. PBMCs were then frozen in an isopropyl alcohol freezing chamber at −80°C overnight before transfer to liquid nitrogen. Frozen samples were shipped to the Center for Disease Control and Prevention (Atlanta, GA), where they were stored in liquid nitrogen until tested.

Induction of vaccine-specific memory B cells (MBCs) was measured by an enzyme-linked immunosorbent assay (ELISPOT) protocol from our laboratory, described previously by Reber et al [15]. Briefly, PBMCs at a concentration of 2×10^6 cells/mL were incubated in complete Roswell Park Memorial Institute basal medium with 10% fetal bovine serum and mitogens. The mitogens included class B CpG oligonucleotides (InvivoGen, San Diego, CA), protein A, and pokeweed mitogen (both from Sigma-Aldrich, St. Louis, MO) at a final concentration of 1 μg/mL. After 6 days of incubation, cells were collected, washed free from the mitogens, and plated onto antigen-coated multiscreen plates at 3-fold serial dilution, starting at 1×10^6–1.5×10^6 cells/well. Inactivated A/California/7/2009(H1N1), and A/Perth/16/2009(H3N2)-like strain monovalent vaccines provided by Dr Michael Decker of Sanofi Pasteur were used for coating plates at concentration 20 μg/mL. Anti-human IgG or anti-human IgA (Southern Biotech) was used at 10 μg/mL to cover plates for total IgG or IgA assessment, respectively. Spot-forming units were counted with the ImmunoSpot system (Cellular Technology, Cleveland, OH) and expressed as the percentage of antigen-specific IgG-expressing or IgA-expressing B cells after normalization to the total number of IgG-expressing or IgA-expressing B cells. A value of 0.005 was assigned for undetectable antibody-secreting cells (ASCs). Ex vivo vaccine-specific plasmablasts were measured in PBMCs collected on days 0 and 7 by use of an ELISPOT similar to the assay described for MBCs but without in vitro stimulation with mitogens. The number of spots was assigned as 1, if none were detected, and normalized to the PBMC count.

Cellular Immune Responses—To examine $CD4^+$ and $CD8^+$ T-cell responses, frozen PBMCs were thawed and rested for 4 hours before being stimulated with the following wild-type virus strains of the vaccine components in the presence of 1 μg/mL anti-CD28/CD49d (BD Biosciences): A/California/7/2009(H1N1) (multiplicity of infection [MOI], 1), A/Perth/16/2009(H3N2) (MOI, 1), or B/Brisbane/60/2008 (64 hemagglutinin [HA] units). After 1 hour of infection, GolgiPlug (1:1000, BD Biosciences) was added, and cells were incubated overnight. Cells were then stained with a Live/Dead Fixable Blue Dead Cell Stain Kit (Invitrogen), followed by surface staining with CD4-AmCyan (eBioscience), CD8-BV605, CD56-Alexa Fluor 700, and CD69-APC-Cy7 and, finally, by intracellular staining with interferon γ (IFN-γ)–phycoerythrin-Cy7, tumor necrosis factor α (TNF-α)–phycoerythrin, and interleukin 2 (IL-2)–Pacific blue after fixation and permeabilization with BD Cytofix/Cytoperm (BD Biosciences). Samples were acquired on a BD LSRII cytometer and analyzed using FlowJo and Simplified Presentation of Incredibly Complex Evaluations (SPICE) software [16]. The gating strategy for T cells is provided in Supplementary Figure 1. The frequencies of cytokine-positive cells were $\log_{10}$ transformed and graphed as geometric mean percentages, after back transformation.

Isolation of Soluble Recombinant HA (rHA)—As described previously [17], cDNA encoding the mature ectodomain from A/California/7/2009(H1N1), A/Perth/16/2009(H3N2), and the HA1 of influenza B/Brisbane/60/2008 were synthesized as codon-optimized constructs (Genscript) and subcloned into the baculovirus transfer vector, pAcGP67-B (BD Biosciences), in frame with an N-terminal baculovirus GP67 signal peptide and a C-terminal thrombin cleavage site, a T4 fibritin sequence for generating functional trimers, and a His-Tag to aid purification of rHA. Secreted soluble rHA proteins were recovered from the cell culture supernatant by tangential flow filtration through a membrane with a molecular weight cutoff of 30 kDa, metal affinity chromatography, and gel filtration chromatography.

Serum HA Binding Avidity—Binding of human sera to rHA was performed by biolayer interferometry on an Octet Red instrument (Pall ForteBio, CA) according to the manufacturer's instructions. Briefly, rHA was bound to anti-penta-His biosensors by incubating biosensors into solutions of rHA (25 μg/mL) in Fortebio's kinetics buffer (phosphate-buffered saline [pH 7.4], 0.01% bovine serum albumin, and 0.002% Tween 20), using a Sidekick offline biosensor immobilization station (Pall ForteBio, Fremont, CA). Receptor-destroying enzyme–treated human study sera were diluted 1:80 with kinetics buffer, and their association/disassociation to rHA-bound biosensors was assessed. Data were analyzed using the system software, and binding results are presented as geometric mean shifts in wavelength (GMWs; in nanometers) at the end of the association step.

Data Analysis

This study assessing CMI responses was not powered for statistical analyses; hence, no statistical analyses were performed. Log_{10}-transformed antibody binding and T- and B-cell responses and avidity were used as dependent variables and summarized as geometric mean percentages (GMPs) after back transformation. Means were estimated using repeated measures linear mixed models. HI titers were log_2 transformed and summarized as geometric mean titers (GMTs) after back transformation. All data shown represent the mean and standard errors of the mean.

RESULTS

Study Participants Characteristics

Seventy-nine of 80 initially targeted HIV-infected and HIV-uninfected participants aged 19–58 years were enrolled in this substudy of CMI responses (Table 1) [11]. Of the HIV-infected individuals enrolled, 38 had advanced HIV disease ($CD4^+$ T-cell count, <200 cells/μL) and 41 participants had mid-stage chronic infection ($CD4^+$ T-cell count, ≥200 cells/μL). No statistical comparisons of any disease characteristics listed were done.

Serological Responses to Vaccination

We measured the HI responses for the cohorts participating in the comprehensive evaluation of CMI responses (Figure 1A). Baseline (ie, prevaccination) HI GMT was low (10; range, 7–11) for all study participants irrespective of their HIV status. Vaccination induced peak HI titers after 1 month, which decreased by 6 months and remained unchanged at 12 months

for all groups. At 1 month, the HIV-uninfected group mounted seroprotective responses (HI titer, ≥40) to all components of the vaccine, with modest humoral responses to the A(H3N2) and B components and more-robust responses to the A(H1N1) component (Figure 1A). In contrast, HIV-infected individuals did not demonstrate any seroprotective responses except against the A(H1N1) vaccine component in the group with a $CD4^+$ T-cell count of ≥200 cells/μL, probably because of poor immunogenicity of the H3N2 and B components (Figure 1A). The group with a $CD4^+$ T-cell count of <200 cells/μL demonstrated only a slight increase in postvaccination HI titers, reaching a maximum HI GMT of 20 for the A(H1N1) component 1 month after vaccination, with no substantial increase in responses to the A(H3N2) or B components of vaccine. Neither HIV status nor route of vaccination had an impact on the induction of seroprotective responses (Figure 1A).

Antibody Affinity Binding

To further understand the effect of vaccination routes on humoral response, the HA-binding avidities of receptor-destroying enzyme–treated sera from the HIV-infected groups and HIV-uninfected cohort were determined by a biolayer interferometry assay. In this assay, binding of serum antibodies to rHA-coated biosensor tips results in an increase in the thickness of the biosensor tip that can be measured as a shift in wavelength. Similar baseline levels of binding avidities for HAs of the 3 vaccine viruses were detected among the HIV-infected and uninfected groups, and binding levels for all 3 HAs were similarly induced 1 month after vaccination (Figure 1B). The route of vaccination did not appear to make a difference, because induced binding responses between intramuscular and intradermal vaccinated groups with or without HIV infection were similar.

Vaccine-Specific Plasmablast Responses

Examination of plasmablast responses revealed low baseline levels for all groups regardless of HIV status (Figure 2). The route of vaccination did not seem to have an impact on the plasmablast responses, because both routes examined had similar levels of plasmablast induction by 7 days (Figure 2A–D). An induction of antigen-specific ASCs after vaccination resulted in an increase IgG-specific and IgA-specific plasmablast responses at day 7 in the HIV-uninfected group and the group with a $CD4^+$ T-cell count of ≥200 cells/μL (Figure 2A–D). IgA-specific plasmablast responses against the A(H1N1) strain increased by about 8-fold and 25-fold following intradermal or intramuscular vaccination, respectively, in the group with a $CD4^+$ T-cell count of ≥200 cells/μL. Against the A(H3N2) strain, these increases were around 10-fold and 11-fold for intradermal and intramuscular vaccine recipients, respectively. We saw similar levels of induction in IgG-specific plasmablasts against the A(H1N1) strain in this group, with fold increases of 35 and 50 following intradermal or intramuscular vaccination, respectively, as well as fold increases of 28 and 51 following intradermal or intramuscular vaccination, respectively, against the A(H3N2) strain. The smallest fold induction in the HIV-uninfected group occurred in the IgA response to intramuscular vaccination, in which a fold increase of 6 was observed in response to the A(H1N1) and A(H3N2) strains. The maximum fold induction in this group was around 90, which was in IgG-specific plasmablasts against the A(H1N1) virus following intradermal vaccination. In the group with a $CD4^+$ T-cell count of <200 cells/μL, IgG-specific and IgA-specific plasmablasts were poorly induced by both the A(H1N1) and A(H3N2) strains, with

the highest increase in induction—around 5-fold—occurring in the IgG-specific plasmablast response to the A(H1N1) strain following intradermal vaccination. No other plasmablast responses in this group were greater than this 5-fold increase.

Vaccine-Specific MBC Responses

HIV infection profoundly affects the MBC population [18]. However, we observed no differences in the antigen-specific MBC responses with respect to the route of vaccination between the HIV-uninfected and infected groups (Figure 3A–D).

The IgA-expressing MBC baseline levels were comparable for all groups (Figure 3A and 3C). IgA-expressing MBCs exhibited a slight increase for both vaccine strains at 1 month in the HIV-uninfected group and the group with a $CD4^+$ T-cell count of ≥200 cells/μL. The group with a $CD4^+$ T-cell count of <200 cells/μL demonstrated no increases in IgA-expressing MBCs in response to vaccination (Figure 3A and 3C).

The baseline level of A(H1N1)-specific IgG-expressing MBCs in the HIV-uninfected group was slightly higher than those in the HIV-infected groups. The baseline level of A(H3N2)-specific IgG-expressing MBCs in the HIV-uninfected group was only slightly higher than those in the group with a $CD4^+$ T-cell count of ≥200 cells/μL. The level of IgG-expressing MBC induction by the A(H1N1) and A(H3N2) vaccine components was more robust in the uninfected group than in the HIV-infected groups 1 and 6 months after vaccination. The group with a $CD4^+$ T-cell count of <200 cells/μL demonstrated a slight increase in IgG-expressing MBCs at 1 and 6 months in response to A(H1N1). Compared with the group with a $CD4^+$ T-cell count of <200 cells/μL, the group with a $CD4^+$ T-cell count of ≥200 cells/μL demonstrated higher increases in IgG-expressing MBCs at 1 month but similar levels at 6 months for both vaccine components. The HIV-uninfected group demonstrated the highest level of IgG-expressing MBC induction among all groups at both time points after vaccination.

$CD4^+$ and $CD8^+$ T-Cell Responses

Triple-cytokine-producing T cells have been shown to be functionally superior to single cytokine producers [19]. After having found no differences in single-cytokine or double-cytokine producers (data not shown) between groups, we examined the distribution of triple-cytokine-producing $CD4^+$ T-cell responses to any virus at various time points after vaccination (Figure 4). We observed no differences between intradermal and intramuscular vaccination in terms of the generation of triple-cytokine-producing cells over time or between groups, although responses appeared to be lowest in the group with a $CD4^+$ T-cell count of <200 cells/μL (Figure 4A and 4B). We saw a similar trend with the $CD8^+$ T-cell responses (Supplementary Figure 2).

We also used SPICE to examine the cytokine profiles of responding $CD4^+$ T cells. The vaccination route did not have any impact on the profile of the responding cells (Supplementary Figure 3). However, 6 months after vaccination, there was a greater proportion of $CD4^+$ T cells secreting only TNF-α among HIV-infected participants in the groups with $CD4^+$ T-cell counts of <200 or ≥200 cells/μL, compared with the HIV-uninfected group. In contrast, in the HIV-uninfected group, we observed expansion of the

proportion of cells secreting only IFN-γ 6 months after vaccination, while this was not clearly observed in the HIV-infected groups. We also observed an increase in the proportion of cells secreting IFN-γ in the HIV-uninfected group following vaccination. This difference was also observed at month 6, although it was only seen in the intramuscular group.

The route of immunization did not change the $CD8^+$ T-cell cytokine profile (Supplementary Figure 4). At 6 months, the $CD8^+$ T-cell compartment among HIV-uninfected participants appeared to show greater representation of cells expressing IFN-γ, compared with the HIV-infected groups, irrespective of $CD4^+$ T-cell count. This difference was also seen on day 7 (data not shown). Overall, we did not observe any correlation between the T-cell responses and the HI titers.

DISCUSSION

In our study, which compared cell-mediated immunity in intramuscular versus intradermal vaccines, the route of vaccination did not have an effect on antibody responses measured by HI titers, antibody avidity, T-cell responses, or B-cell responses. However, the serological and cellular immune responses to influenza vaccination in HIV-infected individuals with a $CD4^+$ T-cell count of <200 cells/μL were poor.

The principal measure of protection from influenza virus infection has remained the HI titer, since it was first demonstrated to reduce susceptibility to challenge, in 1972 [20]. The use of HI titers as end points in determination of vaccine efficacy has persisted owing to the cumbersome nature, costs, and ethical considerations associated with trials relying ultimately on actual influenza virus infection. However, cell-mediated responses could provide better readouts of the vaccine response [21], in addition to being better correlates of protection in certain populations [13, 14]. Although it has been shown that standard-dose intradermal vaccine does not result in higher HI titers than standard-dose intramuscular vaccine in HIV-infected individuals [11], assessment of the cell-mediated responses remain lacking.

In our study, serological responses measured by HI titers suggest that the A(H1N1) component of the vaccine appears to be more immunogenic than the A(H3N2) and B components. Postvaccination serological responses were affected by HIV infection status and sharply decreased for the group with a $CD4^+$ T-cell count of <200 cells/μL, because seroprotective titers were not achieved for all vaccine components. In contrast, seroprotective titers were not induced in patients with a $CD4^+$ T-cell count of ≥200 cells/μL to the A(H3N2) and B components of vaccine, perhaps owing to their poor immunogenicity. The binding avidities of sera obtained 1 month after vaccination from all groups were similar, and the route of vaccination had no impact, although the binding avidities increased after vaccination. In the current study, we also investigated influenza virus–specific IgA-expressing and IgG-expressing MBC acute and memory responses. Influenza virus–specific IgG-expressing ASCs were detected at higher levels than IgA-expressing ASCs in peripheral blood for both immunization routes. HIV status severely affected influenza virus–specific plasmablast responses to vaccine in the group with a $CD4^+$ T-cell count of <200 cells/μL,

while plasmablast responses were comparable between the HIV-uninfected group and the group with a $CD4^+$ T-cell count of ≥200 cells/μL.

The influenza virus–specific MBC response (especially the IgG-expressing MBC response) to vaccine was highly dependent on HIV status. The baseline levels of IgG-expressing MBCs in the HIV-uninfected group were higher than those in the HIV-infected groups, and the level of induction in the HIV-uninfected group was also more robust than in the HIV-infected groups. The group with a $CD4^+$ T-cell count of <200 cells/μL demonstrated only a slight increase in IgG-expressing MBCs in response to vaccine. Compared with the group with a $CD4^+$ T-cell count of <200 cells/μL, the group with a $CD4^+$ T-cell count of ≥200 cells/μL showed higher expansion of IgG-expressing MBCs at 1 month but a similar rate of waning by month 6 for both vaccine components. These results indicated that HIV infection affected the available MBC pool and the duration of the immune response at a higher level than MBC expansion in response to vaccination. This suggests the selective depletion of cross-reactive MBCs, as well as those that recognize historical influenza virus strains, which has previously been shown by Wheatly et al [22].

Overall, the HIV-infected group with advanced disease demonstrated severely diminished HI titers and acute and memory responses. There was no statistical difference in acute or memory responses detected between intramuscular and intradermal groups among infected and uninfected participants.

Influenza vaccine–specific $CD4^+$ T-cell responses have been shown to accurately predict the rise in HI titers [23, 24]. While T-cell immunity does not prevent initial infection, it does inversely correlate with disease severity, making the study of T-cell responses important in vaccine evaluation [25–29]. In our studies, we examined polyfunctional T-cell responses to in vitro stimulation with wild-type viruses. Although the study is not powered for statistical analyses to draw conclusions, we observed phenotypic changes in the $CD4^+$ T-cell profile in healthy individuals, demonstrated by a majority of cells producing IFN-γ, whereas most HIV-infected individuals seemingly increased production of TNF-α regardless of $CD4^+$ T-cell count. However, the route of vaccination did not affect these differences.

Immune responses to influenza vaccine were severely compromised in the group with a $CD4^+$ T-cell count of <200 cells/μL; therefore, approaches that restore the $CD4^+$ T-cell subset to >200 cells/μL before influenza vaccination or facilitate passive transfer of influenza virus–specific human polyclonal antibodies or a cocktail of influenza virus–specific human monoclonal antibodies during influenza season could protect patients with advanced HIV disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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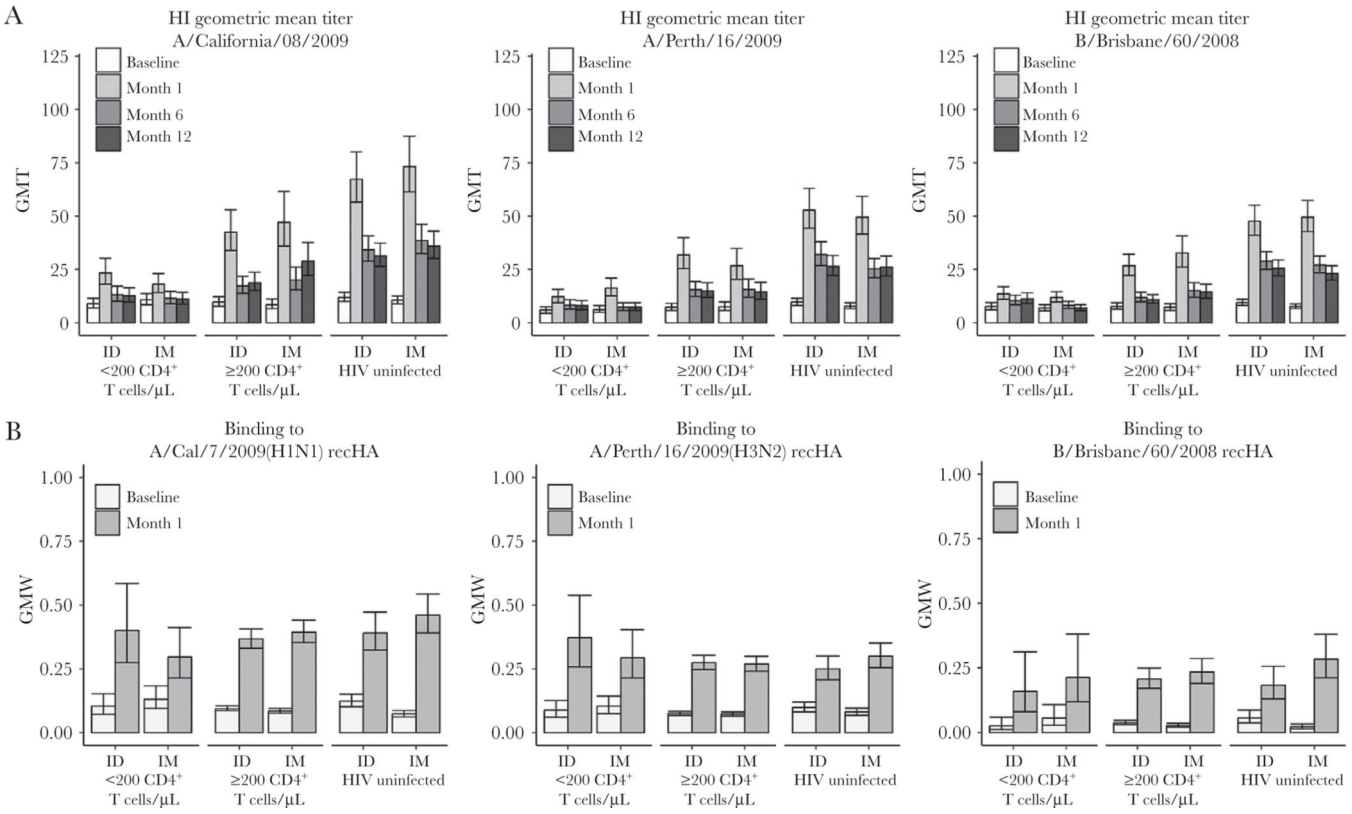

Figure 1.

Hemagglutination-inhibition (HI) titers and antibody affinity binding. *A*, Serum HI antibody titers specific for influenza A(H1N1), A(H3N2), and B vaccine components were assessed on day 0, month 1, month 6, and month 12 after vaccination for human immunodeficiency virus (HIV)–infected individuals ($CD4^+$ T-cell count, <200 or ≥200 cells/μL) and HIV-uninfected individuals. *B*, Hemagglutinin (HA)–binding avidities of sera from the HIV-infected and HIV-uninfected groups were analyzed by biolayer interferometry, using biosensors loaded with recombinant HAs of the vaccine viruses, A/California/7/2009(H1N1), A/Perth/16/2009(H3N2), and B/Brisbane/60/2008. Geometric means of shifted wavelength (in nanometers) as a result of antibody-antigen interaction are shown for HIV-infected individuals ($CD4^+$ T-cell count, <200 or ≥200 cells/μL) and HIV-uninfected individuals. GMW, geometric mean wavelength; ID, intradermal; IM, intramuscular.

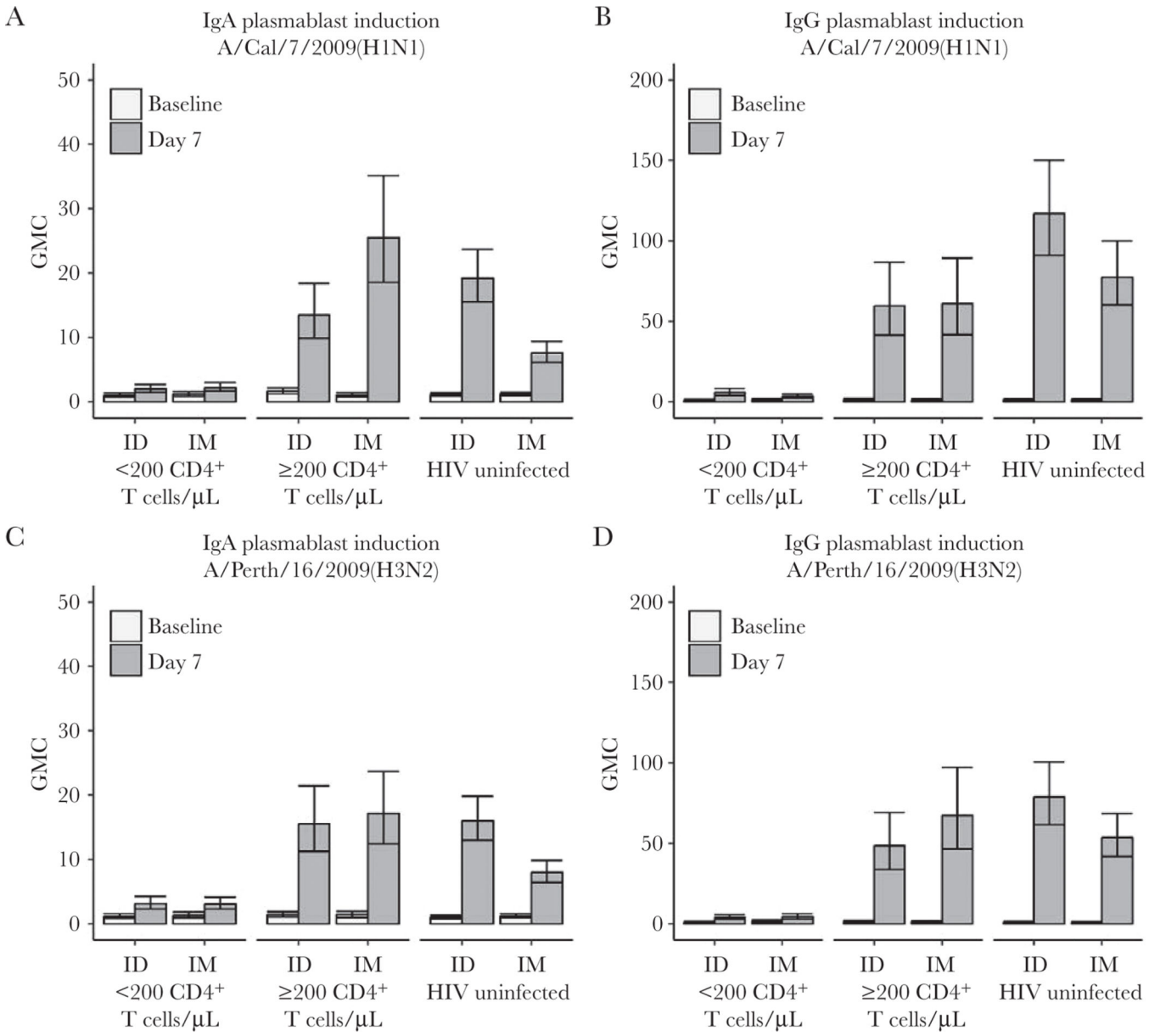

Figure 2.

Vaccine-specific plasmablast responses. *A* and *C*, The geometric mean count (GMC)/10^6 peripheral blood mononuclear cells (PBMCs) of immunoglobulin A (IgA)–expressing plasmablasts (PBs) specific for influenza A(H1N1) (*A*) and A(H3N2) (*C*) vaccine components were assessed by enzyme-linked immunospot (ELISPOT) assay for human immunodeficiency virus (HIV)–infected individuals ($CD4^+$ T-cell count, <200 or ≥200 cells/μL) and HIV-uninfected individuals at days 0 and 7 after intramuscular (IM) or intradermal (ID) vaccination. *B* and *D*, The number of immunoglobulin G (IgG)–expressing PBs specific for influenza A(H1N1) (*B*) and A(H3N2) (*D*) vaccine components were assessed by ELISPOT assay for HIV-infected individuals ($CD4^+$ T-cell count, <200 or ≥200 cells/μL) and HIV-uninfected individuals at days 0 and 7 after IM or ID vaccination.

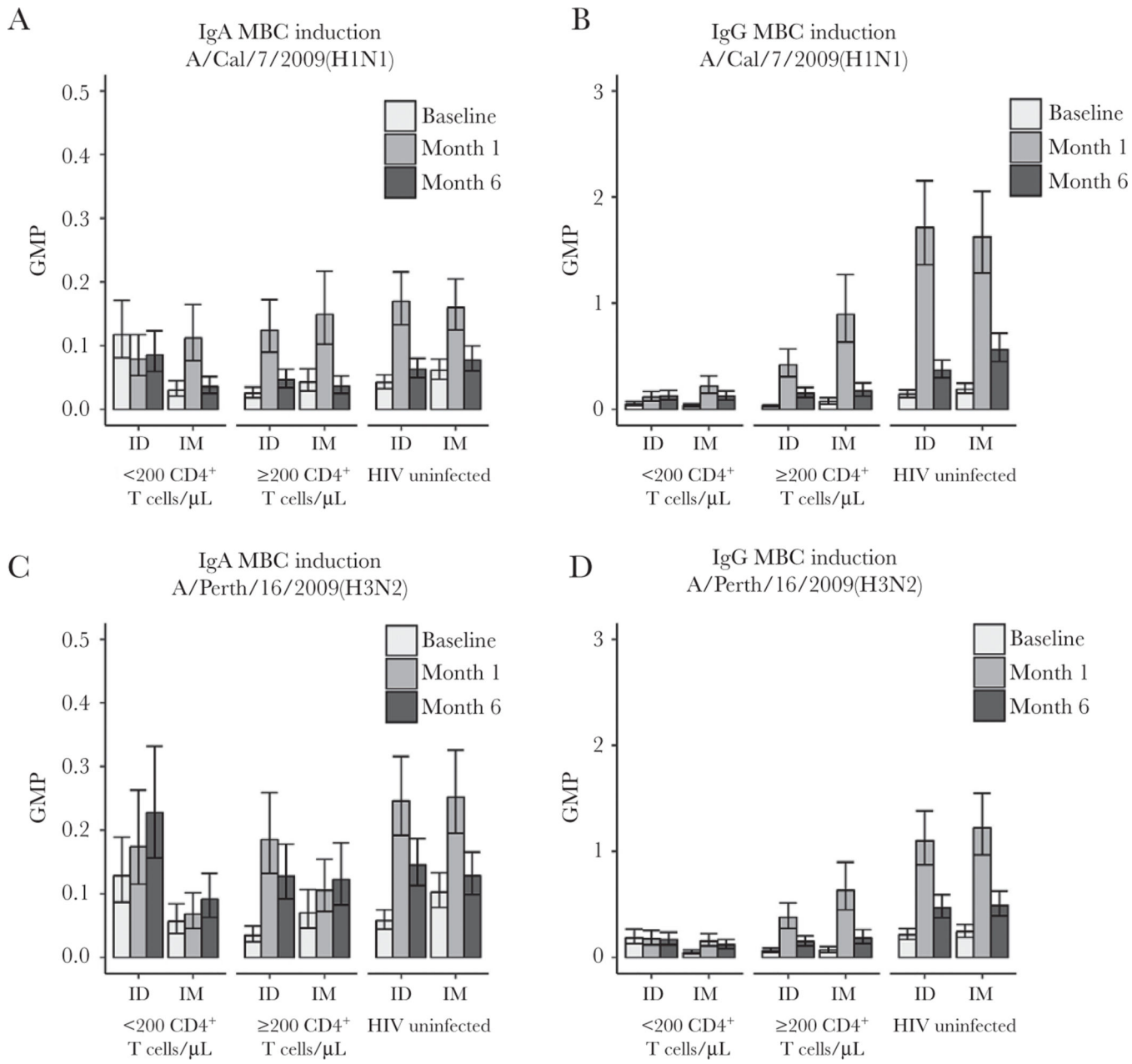

Figure 3.

Vaccine-specific memory B-cell responses. *A* and *C*, Geometric mean percentages (GMPs) of immunoglobulin A (IgA)–expressing memory B cells specific for influenza A(H1N1) (*A*) and A(H3N2) (*C*) vaccine components were assessed by enzyme-linked immunospot (ELISPOT) assay for human immunodeficiency virus (HIV)–infected individuals ($CD4^+$ T-cell count, <200 or ≥200 cells/μL) and HIV-uninfected individuals at 0 days, 1 month, and 6 months after intramuscular (IM) or intradermal (ID) vaccination. *B* and *D*, The number of immunoglobulin G (IgG)–expressing memory B cells specific for influenza A(H1N1) (*B*) and A(H3N2) (*D*) vaccine components were assessed by ELISPOT assay for HIV-infected

individuals ($CD4^+$ T-cell count, <200 or ≥200 cells/μL) and HIV-uninfected individuals at 0 days, 1 month, and 6 months after IM or ID vaccination.

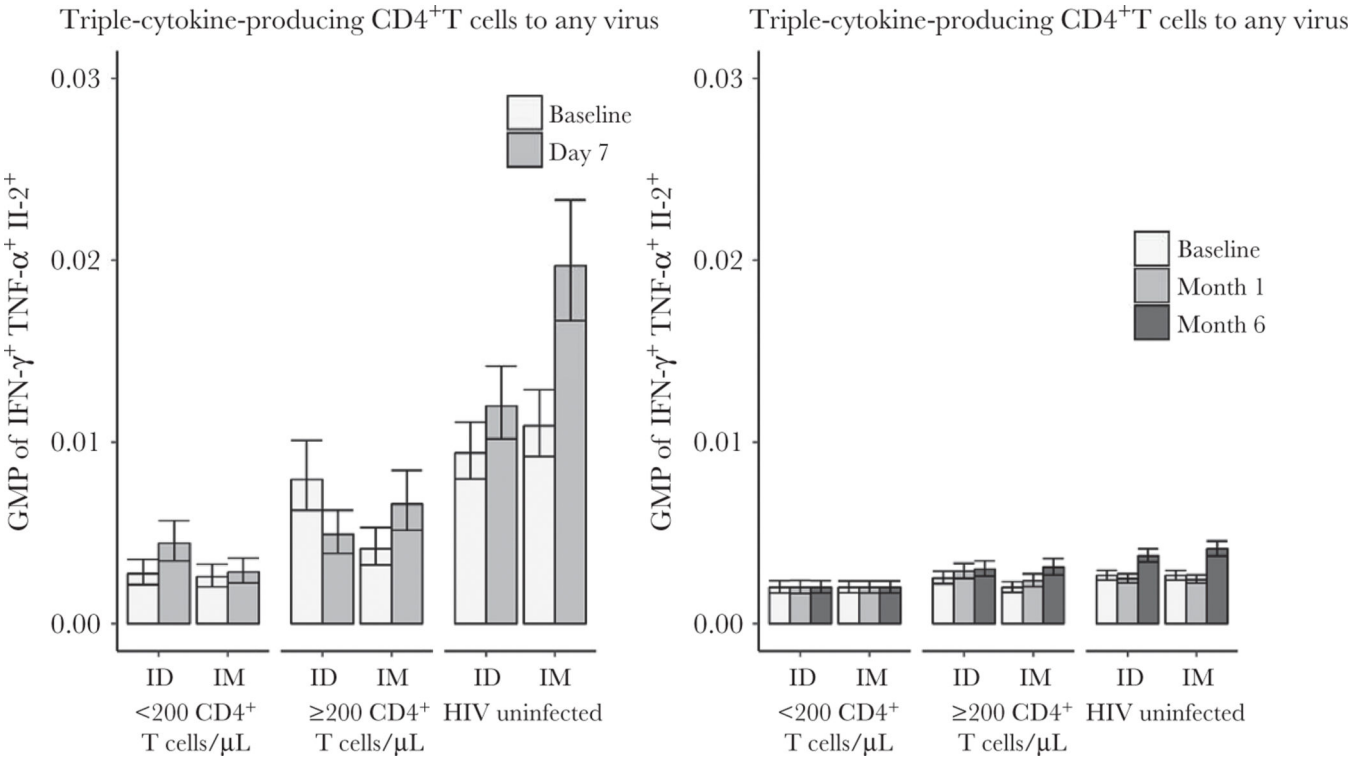

Figure 4.

Multifunctional $CD4^+$ T-cell responses. Changes in the interferon γ (IFN-γ), tumor necrosis factor α (TNF-α), and interleukin 2 (IL-2) responses among $CD4^+$ T cells was assessed by overnight in vitro stimulation with A/California/7/2009(H1N1), A/Perth/16/2009(H3N2), and B/Brisbane/60/2008 on days 0 and 7 (*A*), or day 0, month 1, and month 6 (*B*) after vaccination with either intramuscular (IM; Vaxigrip) and intradermal (ID; Intanza/Idflu) vaccines. Data are expressed as geometric mean percentages (GMPs) after $\log_{10}$ transformation of the combined cytokine response to all viruses. Triple-cytokine producers were estimated using Boolean gating on FlowJo.

Table 1.

Baseline Demographic and Clinical Characteristics of Study Participants

Characteristic	HIV Infected, CD4+ T-Cell Count <200 cells/μL		HIV Infected, CD4+ T-Cell Count ≥200 cells/μL		HIV Uninfected	
	Intramuscular (n = 20)	Intradermal (n = 18)	Intramuscular (n = 17)	Intradermal (n = 24)	Intramuscular (n = 39)	Intradermal (n = 40)
Age, y, median (range)	28 (21–41)	29 (19–45)	27 (21–58)	28 (20–38)	30 (19–48)	29 (20–45)
Medical condition, no. (%)						
Hepatitis B	2 (10)	0 (0)	0 (0)	2 (8.3)	1 (2.6)	0 (0)
Hepatitis C	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Tuberculosis	2 (10)	1 (5.6)	0 (0)	1 (4.2)	0 (0)	0 (0)
Asthma	0 (0)	0 (0)	0 (0)	1 (4.2)	1 (2.6)	1 (2.5)
Diabetes	0 (0)	0 (0)	0 (0)	1 (4.2)	0 (0)	0 (0)
Chronic lung disease	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.6)	2 (5)
Cardiovascular disease	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
HIV infection duration, y, median (range)	3.7 (0.1–12.1)	1.3 (0.1–6.7)	0.9 (0.4.8)	2.2 (0.1–25.3)	0	0
Known nadir CD4+ T-cell count, no. (%)[a]						
<200 cells/μL	20 (100)	18 (100)	2 (11.8)	6 (25)	NA	NA
≥200 cells/μL	0 (0)	0 (0)	10 (58.8)	16 (66.7)	NA	NA
Missing	0 (0)	0 (0)	5 (29.4)	2 (8.3)	NA	NA
Detectable HIV RNA load at study enrollment, no. (%)	17 (85)	14 (77.8)	15 (88.2)	20 (83)	NA	NA
Receiving ART, no. (%)	10 (50)	11 (61.1)	4 (23.5)	8 (33.3)	NA	NA
ART duration, y, median (range)	0.5 (0.01–2.19)	0.4 (0.002–5.7)	0.1 (0.005–3.0)	0.7 (0.06–5.85)	NA	NA

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; NA, not applicable; y, years.

[a]The nadir CD4+ T-cell count (or the lowest count since diagnosis of HIV infection) was self-reported for this study. If a participant did not know their nadir CD4+ T-cell count, they had the option to reply "I don't know." Among HIV-infected patients with CD4+ T-cell counts ≥200, nadir CD4+ T-cell counts for 5 individuals who received intramuscular vaccine and 2 individuals who received intradermal vaccine were unknown.