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Th Cell-Independent Immune Responses to Chimeric Hemagglutinin/Simian Human Immunodeficiency Virus-Like Particles Vaccine[1]

Qizhi Yao,[2]* Rongxin Zhang,* Lizheng Guo,† Min Li,* and Changyi Chen*

CD4$^+$ Th cells are believed to be essential for the induction of humoral and cellular immune responses. In this study we tested the effect and possible mechanisms of the major antigenic component in influenza, hemagglutinin (HA), in helping HIV Env to induce immune responses in CD4$^+$ T cell knockout (CD4 KO) mice. Simian HIV virus-like particles (SHIV VLPs) or phenotypically mixed chimeric influenza HA/SHIV VLPs were used as immunogens to immunize CD4 KO mice either i.p. or intranasally (i.n.). We found that chimeric HA/SHIV VLPs significantly induced a greater IgG Ab response in both i.p. and i.n. immunized mice and a greater IgA Ab response in mucosal washes in i.n. immunized mice compared with SHIV VLPs. Importantly, chimeric HA/SHIV VLPs induced ~3-fold higher neutralizing Ab titers against HIV 89.6 than SHIV VLPs in the absence of CD4$^+$ T cell help. There was also ~40% more specific lysis of the HIV Env-expressing target cells in chimeric HA/SHIV VLP-immunized than in SHIV VLP-immunized CD4 KO mouse splenocytes. Moreover, we have found that chimeric HA/SHIV VLPs could efficiently bind and activate dendritic cells and stimulate the activated dendritic cells to secret TNF-α and IFN-γ. Therefore, chimeric HA/SHIV VLPs could efficiently prime and activate APCs, which could, in turn, induce immune responses in a CD4$^+$ T cell-independent manner. This study suggests a novel adjuvant role of influenza HA as well as a new strategy to develop more effective therapeutic vaccines for AIDS patients with low CD4$^+$ T cell counts. *The Journal of Immunology,* **2004, 173: 1951–1958.**

One of the major effects during the course of HIV infection is the gradual loss of CD4$^+$ T cells. Therefore, it is important for a therapeutic AIDS vaccine to elicit robust immunity in the absence of CD4$^+$ T cells. Usually, CD4$^+$ T cells are believed to play a critical role in the regulation of Ig class switching as well as CTL activity during the immune response to specific Ags (1–4). CD4$^+$ T cells could cognately help B cells by promoting B cell activation and Ig isotype switch via direct T-B cell interaction. CD4$^+$ T cells could also furnish noncognate or bystander help to B cells and CD8$^+$ T cells via cytokines secreted by the activated CD4$^+$ T cells. Alternatively, the help could be delivered by activated APCs, which can directly prime CTL (5). However, some studies have shown that CD4$^+$ T cell-independent CTL or Ig class switching exists (6–9). In $\alpha\beta^+$ T cell-deficient mice, IgG responses could be induced by infection of live vesicular stomatitis virus or recombinant vaccinia virus expressing the vesicular stomatitis virus gp, but not by immunization with formalin-inactivated virus (10–12). Immunization with inactivated influenza virus can induce IgG responses in mice without CD4$^+$ T cells (13). Furthermore, a study with recombinant porcine parvovirus virus (PPV)[3]-like particles (PPV-VLP) has demonstrated that dendritic cells (DCs) have the capacity to transfer VLPs to the cytosolic pathway and present these exogenous Ags to CD8$^+$ T cells in a Th cell-independent manner (14). Therefore, it remains to be determined whether CD4$^+$ T cells are required for inducing humoral and cellular immune responses in many vaccine studies.

Many reports have clearly demonstrated that VLPs have great potential to induce both humoral and cellular immune responses and are promising vaccine candidates (15–20). VLPs have also been proven to be potent CTL inducers compared with other vectors (21–24). A study of PPV-VLPs immunization showed a safe and efficient strategy to induce CTLs and stimulate antiviral immunity in a CD4-independent manner (22). These recombinant pseudoparticles represent a promising strategy to present several CTL epitopes from the same or different viral proteins to the immune system. Previous investigations have demonstrated that CpG-conjugated gp120 (25) or OVA (26) induces equivalent CTL responses in CD4 knockout (KO) and wild type (w.t.) mice, whereas the CTL response in CD4 KO mice immunized with a mixture of CpG and proteins is reduced. We have previously shown that immunization with chimeric hemagglutinin (HA)/simian HIV (SHIV) VLPs in w.t. mice induced higher HIV Env-specific humoral as well as cellular immune responses than immunization with SHIV VLPs. We have proposed a possible adjuvant effect of HA incorporated into SHIV VLPs (20). Therefore, we explored the adjuvant effect of HA on chimeric HA/SHIV VLPs on systemic and mucosal immunization in CD4 KO mice.

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[3] Abbreviations used in this paper: PPV, porcine parvovirus; BMDC, mouse bone marrow-derived DC; CBA, cytometric bead array; DC, dendritic cell; HA, hemagglutinin; HA/SHIV VLP, phenotypically mixed chimeric influenza HA/SHIV VLP; i.n., intranasal immunization route; KO, knockout; m.o.i., multiplicity of infection; rBV, baculovirus recombinant; SHIV, simian HIV; VLP, virus-like particle; vv, vaccinia virus; w.t., wild type; sMAGI, simian multinuclear activation of a galactosidase indicator.

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In this study we compared the immunogenicities of SHIV VLPs and chimeric HA/SHIV VLPs and investigated the induction of systemic and mucosal humoral immune responses in serum and mucosal secretions in $CD4^+$ T cell-deficient mice by both systemic and mucosal immunizations. In addition, we have determined the neutralizing Ab production and CTL activity by different immunogens and different immunization routes. Furthermore, we have investigated the action of VLPs on DCs, which could explain the adjuvant effect of HA in inducing IgG Ab and CTL responses in the absence of $CD4^+$ T cell help.

Materials and Methods

Cells, proteins, and Abs

Spodoptera frugiperda Sf 9 cells were maintained in suspension in serum-free SF900 II medium (Invitrogen Life Technologies, Grand Island, NY) in Spinner flasks. EL4 cells were purchased from American Type Culture Collection (Manassas, VA) and maintained in RPMI 1640 with 10% FCS at 37°C with 5% CO_2. HeLa cells were maintained in 1× DMEM with 10% FCS at 37°C with 5% CO_2.

A vaccinia virus recombinant expressing HIV truncated Env protein (vv-HIV gp160t) was generated previously (27). The HIV gp160t protein was purified from vv-HIV gp160t-infected HeLa cell lysates using a lectin column (Sigma-Aldrich, St. Louis, MO) and used to coat ELISA plates. Briefly, HeLa cells were infected with vv-HIV gp160t at a multiplicity of infection (m.o.i.) of 3 for 36 h. Cells were then lysed with radioimmunoprecipitation assay buffer containing a protease inhibitor tablet (Roche Diagnostic Systems, Somerville, NJ). After equilibrating the column, 50 ml of the sample was loaded; after the washing buffer, an elution buffer was applied, and fractions were collected and analyzed by a Bio-Rad protein assay (Hercules, CA) to determine the protein concentration. The purity and identity of the purified proteins were detected by Coomassie Blue staining and Western blot analysis as described previously (28). The purity of this protein was ~95% as shown by Coomassie Blue staining. Purified mouse IgG, IgG1, and IgG2a for ELISA were purchased from Southern Biotechnology Associates (Birmingham, AL).

Production of SHIV VLPs and phenotypically mixed chimeric HA/SHIV VLPs

The production and purification of SHIV VLPs as well as chimeric HA/SHIV VLPs were previously described in detail (20, 28). Briefly, Sf9 insect cells were coinfected with a baculovirus recombinant (rBV) expressing SIVmac239 *gag* at an m.o.i. of 2 and an rBV expressing HIV env_t (a cytoplasmic domain truncation mutant of Env) at an m.o.i. of 10. For phenotypically mixed HA/SHIV VLPs, Sf9 cells were coinfected with rBV SIV *gag* at an m.o.i. of 2, rBV HIV env_t at an m.o.i. of 10, and rBV HA at an m.o.i. of 5. At 3 days postinfection, the culture media were collected and centrifuged at 2500 rpm for 20 min (GPR desktop centrifuge; Beckman Coulter, Fullerton, CA). The supernatant was then filtered through a 0.45-μm pore size filter, and VLPs were pelleted at 120,000 × *g* for 2 h at 4°C. The resuspended VLPs were purified through a 20–60% continuous sucrose gradient at 100,000 × *g* for 16 h at 4°C. The VLP band was collected and dialyzed against PBS using a 10,000 m.w. cut-off membrane. VLPs were then pelleted and resuspended overnight in PBS. Western blot analysis with primary Ab against HIV Env was performed to determine Env incorporation into the VLPs, and primary Ab against influenza A/PR8 virus was used to determine HA incorporation into the VLPs. The total protein concentration of VLPs was determined by Bio-Rad protein assay. The endotoxin level in each SHIV VLPs preparation was quantitated with *Limulus* amebocyte assay (Associates of Cape Cod, Woods Hole, MA) and was controlled <0.0041 μg/ml.

Immunizations

C57BL/6-$Cd4^{tmlMak}$ mice, which had a targeted disruption in their CD4 gene and therefore lacked functional $CD4^+$ T cells (29) (The Jackson Laboratory, Bar Harbor, ME; 50 μg/mouse, five mice per group), were immunized intranasally (i.n.) or i.p. with SHIV VLPs or chimeric HA/SHIV VLPs for 0, 2, 4, and 6 wk. The i.n. immunization was performed with a 10-μl Eppendorf pipette tip to deliver VLPs to both nares of a mildly anesthetized mouse. The drops were placed into the nares at 8 μl/administration four times in 15 min (maximum volume, 32 μl). In addition, two groups (n = 5/group) of w.t. C57BL/6J mice (The Jackson Laboratory) were used for the same i.n. immunization.

Sample collection

Blood samples were collected by retro-orbital plexus puncture at 0, 2, 4, 6, and 8 wk. After clotting and centrifugation, serum samples were collected. Vaginal lavages were collected by washing the vagina with 100 μl of PBS at 8 wk. Feces were collected at 8 wk, and IgA was extracted by routine methods. Briefly, five to eight pieces of freshly voided feces were collected, weighed, and resuspended in PBS at a ratio of 5 μl/mg feces to standardize for variability in the amount of fecal material collected. The solid matter was resuspended by vortexing vigorously until solutions were homogenous. Samples were then spun in a microcentrifuge for 10 min, and supernatants were collected. After collection, 1% (v/v) 100 mM PMSF in isopropanol was added to each mucosal sample as a protease inhibitor. All samples were stored at −20°C until analysis for Ab titration. Spleens were harvested at 8 wk and ground to make single-cell suspensions of splenocytes (25).

ELISA

All sera and mucosal secretions were individually collected, and IgG, IgG1, IgG2a, and IgA Ab titers to HIV Env were determined by ELISA. Immunlon-4 HBX 96-well microtiter plates (Nunc Life Technologies, Basel, Switzerland) were coated with 100 μl of purified HIV Env protein (2 μg/ml)/well in borate-buffered saline at 4°C overnight. Plates were blocked with PBS containing 1% BSA at room temperature for 2 h. After three washes in PBS containing 0.05% Tween 20, 100-fold diluted sera or 5-fold diluted mucosal secretion samples were added to the wells and incubated at 4°C overnight. After four washes, the wells were treated with goat anti-mouse IgG, IgG1, IgG2a, or IgA-peroxidase conjugates (Sigma-Aldrich) for 1 h at room temperature. After removal of the unbound conjugates, the substrate solution prepared in ABTS (Sigma), and hydrogen peroxide was added to the plates. ODs were then read at 405 nm in an ELISA reader (EL 311; Bio-Tek Instruments, Winooski, VT). Concentrations of IgG, IgG1, IgG2a, and IgA were determined by comparing the reading for the experimental samples with the standard curves. Data were analyzed by using DeltaSOFT II program (Biometallics, Princeton, NJ). Results are given as the arithmetic mean ± SD. Ab levels between two groups were compared using Student's *t* test in the Excel program. Significant differences were defined as $p < 0.05$.

Neutralization assay

Neutralization assay was performed using simian multinuclear activation of a galactosidase indicator (sMAGI) cells in a 96-well plate as described by Chackerian et al. (30). HIV stocks were grown in HUT78 cells obtained from American Type Culture Collection. Briefly, 8 wk serum samples were heat-inactivated at 56°C for 1 h and then serially diluted 2-fold in complete DMEM growth medium. The serum sample (25 μl) was added to an equal volume of virus stocks diluted in complete DMEM to contain 100–200 infectious particles. The virus/serum mixture was incubated at 37°C for 1 h and then added to sMAGI cells with DEAE-dextran to a final concentration of 15 μg/ml. After 2-h incubation, an additional 200 μl of complete DMEM was added. After 24 h, the medium was replaced by complete DMEM containing 50 μM AZT (Sigma-Aldrich). Three days after infection, the medium was removed, and the cells were fixed and stained. The neutralization titers were expressed as the highest dilution of serum that reduced the number of blue cells by at least 50%. Preimmune serum was used as the control.

CTL assay

Splenocytes were freshly isolated and used as effector cells in the CTL assay. EL-4 cells ($H\text{-}2^b$) infected with vv-HIV Env at an m.o.i. of 5 were used as target cells. EL-4 cells infected with w.t. vaccinia virus at an m.o.i. of 5 were used as negative control target cells. Three different E:T cell ratios (50:1, 17:1, and 6:1) were tested in the assay with 1×10^4 target cells. The specific lysis of target cells was measured using the Cytotox 96 assay kit according to the manufacturer's instruction (Promega, Madison, WI).

DC preparation

Mouse DCs were derived from C57BL/6J mouse bone marrow as described previously (31). Briefly, bone marrow was obtained from tibia and femurs by flushing them with medium. After lysis of RBC, the cell suspension was treated with a mixture of lineage-specific mAbs to remove non-DCs. The cells were then resuspended in RPMI 1640 supplemented with 5% FBS, L-glutamine (2 mM), penicillin/streptomycin (50 U/ml), 10% nonessential amino acids, and 50 mM 2-ME plus 1000 U/ml recombinant GM-CSF and 1000 U/ml IL-4 (R&D Systems, Minneapolis, MN). Four milliliters of cells were then plated into each well of the six-well plate

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and incubated at 37°C with 5% CO_2. Fresh medium supplemented with GM-CSF and IL-4 was added every 2 days.

VLP binding assay

Mouse bone marrow-derived DCs (BMDC) were incubated with 10 μg of SHIV VLPs or chimeric HA/SHIV VLPs per 10^6 DCs in 100 μl at 4°C for 1 h. After extensive washing, monkey anti-SIV Ab (provided by P. Marx, Aaron Diamond AIDS Research Center, New York, NY) at 1/500 dilution was added and incubated for 30 min at 4°C. Secondary anti-monkey Ab conjugated with FITC at a 1/500 dilution was then stained for another 30 min at 4°C. The cells were fixed with 2% paraformaldehyde and analyzed by FACSCalibur (BD Biosciences, San Jose, CA).

DC activation assay

To evaluate DC maturation, DCs on day 6 were collected and incubated with VLPs at a concentration of 10 μg/10^6 cells in 1 ml of PBS/1% FBS for 1 h at 37°C. VLPs heated for 10 min at 95°C were used as negative controls. LPS at 10 μg/ml was used as a positive control. Untreated DC culture was also used as another negative control. The cells were subsequently incubated for 2 days in 3 ml of RPMI 1640 medium supplemented with 1000 U/ml recombinant human GM-CSF and 1000 U/ml IL-4. At the end of 2-day incubation, cells were harvested, washed with PBS/1% FBS, and subsequently stained with CD11c-PE and each of the DC activation markers (CD40, CD80, CD83, CD86, CD54, MHC class I H2 K^b, and MHC class II I-A^b) labeled with FITC in 100 μl of PBS/1% FBS for 30 min at 4°C. The cells were washed with 3 ml of PBS and then fixed with 2% paraformaldehyde for FACS analysis (FACSCalibur; BD Biosciences). PE- and FITC-labeled DCs were acquired by CellQuest software. CD11c-positive cells were then gated for further analysis.

Cytometric bead array (CBA) for cytokine detection

Cytokines IL-2, IL-5, IL-10, TNF-α, and IFN-γ were detected using the Mouse Th1/Th2 Cytokine CBA kit (BD Cytometric Bead Array; BD Pharmingen, San Diego, CA). Briefly, 50 μl of cell culture supernatant from differently treated DCs was incubated with 50 μl of a mixture of six human cytokine capture beads and 50 μl of PE detection reagent for 3 h at room temperature. Ten different dilution standards and negative controls were performed at the same time. Samples were then washed with 1 ml of washing buffer. Finally, 300 μl of washing buffer was added to each assay tube, and samples were assayed using FACSCalibur. The results were analyzed using BD CBA software version 1.1 (BD Biosciences).

Results

Chimeric HA/SHIV VLPs induce CD4$^+$ T cell-independent IgG responses

To investigate the potential of chimeric HA/SHIV VLPs to induce IgG responses to HIV Env in the absence of CD4$^+$ T cells, four groups (n = 5 mice/group) of CD4$^+$ T cell-deficient mice were used for both systemic (i.p.) and mucosal (i.n.) immunizations. In addition, two groups (n = 5/group) of w.t. C57BL/6J mice were used for the same i.n. immunization procedures. The magnitude of HIV Env-specific IgG responses to either i.n. or i.p. immunization with SHIV VLPs or chimeric HA/SHIV VLPs in CD4 KO mice and w.t. C57BL/6J mice was evaluated by quantitative ELISA (Fig. 1*A*). Both SHIV VLPs and chimeric HA/SHIV VLPs were found to induce HIV Env-specific IgG Abs in CD4 KO mice, indicating that CD4$^+$ T cell-independent Ab class switching from IgM to IgG took place after the immunization. Although chimeric HA/SHIV VLPs immunization showed significantly higher IgG production in both routes than SHIV VLPs immunization ($p <$ 0.05), the enhancement of IgG production by the i.p. route (4-fold increase) was more significant than that by the i.n. route (2-fold increase). However, the magnitude of HIV Env-specific IgG Ab induction was lower in CD4 KO mice than in w.t. mice by the mucosal route of immunization (Fig. 1*A*). These data suggest that chimeric HA/SHIV VLPs have the potential to enhance HIV Env-specific IgG Ab production independent of CD4$^+$ Th cells.

To further characterize Ab induction by different VLPs, serum IgG subtypes IgG1 and IgG2a were determined (Fig. 1*B*) in CD4

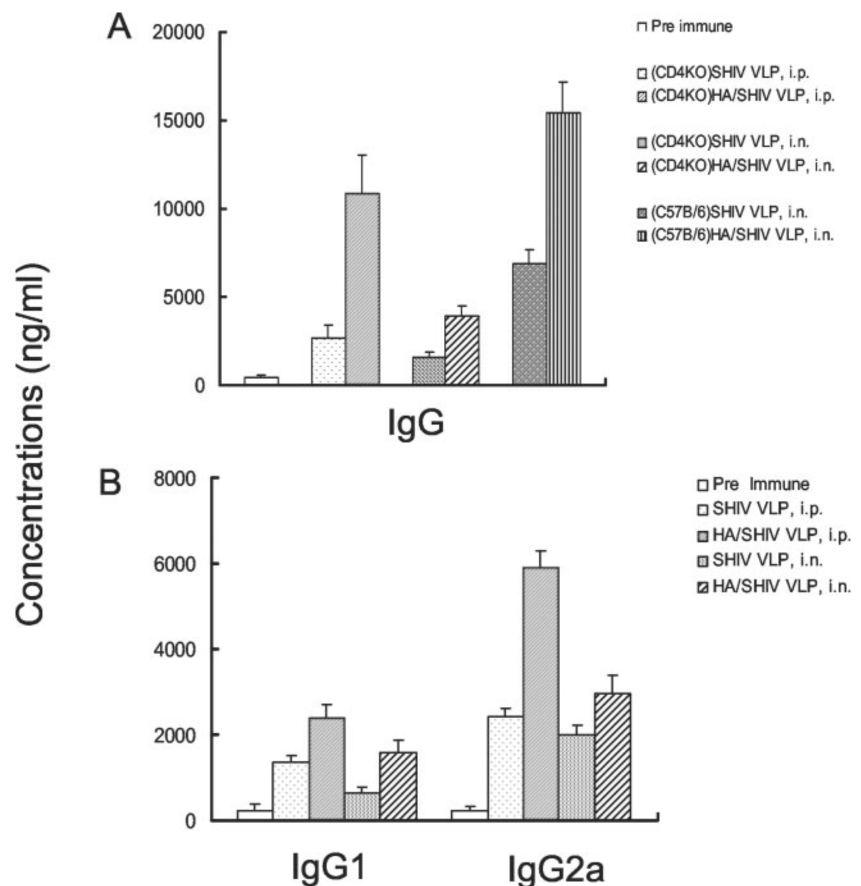

FIGURE 1. Systemic humoral responses against HIV Env after i.p or i.n. immunization with SHIV VLPs or chimeric HA/SHIV VLPs in CD4$^+$ T cell-deficient mice. CD4 KO mice were immunized i.p. or i.n. with 50 μg of VLPs/mouse. The mice were boosted with the same dose every 2 wk, three times (2, 4, and 6 wk). Sera were collected at 8 wk, and the production of IgG as well as the IgG subtypes, IgG1 and IgG2a, against HIV Env protein were measured by ELISA. Results are expressed as the arithmetic mean ± SD from five mice per group. *A*, Serum total IgG Ab production. As a comparison, serum anti-HIV Env IgG production by i.n. immunization with different VLPs in w.t. C57BL/6 was also shown. *B*, Serum IgG subtypes IgG1 and IgG2a production in CD4 KO mice. Pre immune, serum from unimmunized CD4 KO mice.

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KO mice. The quantity of IgG2a was significantly higher than that of IgG1 in all chimeric HA/SHIV VLP-immunized groups ($p < 0.05$). However, there was only a low degree of enhancement of IgG2a production over IgG1 in the SHIV VLPs group. Consistent with our previous results, there was more significant enhancement in i.p. than in i.n. immunized groups. Therefore, this suggests that HA/SHIV VLPs could induce mixed subtypes of IgG Ab responses in CD4 KO mice.

Intranasal immunization with chimeric HA/SHIV VLPs enhances mucosal IgA production in CD4$^+$ T cell-deficient mice

To determine the mucosal immunogenicity of chimeric HA/SHIV VLPs, we detected the level of IgA at different mucosal sites. Both fecal extract and vaginal lavage samples were collected 2 wk after the last boost, and quantitative ELISA were used to determine IgA production by both mucosal and systemic VLPs immunizations. As shown in Fig. 2, mucosal IgA Ab was detected in all i.n. VLP-immunized groups, but not in the i.p. immunized groups. Significantly higher levels of IgA Abs were produced in the chimeric HA/SHIV VLPs i.n. immunized groups in both fecal wash and vaginal lavage ($p < 0.05$). These results demonstrate that VLPs administered i.n. to CD4$^+$ T cell-deficient mice can also induce HIV Env-specific IgA Ab responses at different mucosal sites.

Immunization with chimeric HA/SHIV VLPs in CD4$^+$ T cell-deficient mice induces neutralizing Abs

To explore whether immunization with chimeric HA/SHIV VLPs in CD4 KO mice could induce virus-neutralizing activity in vitro, 50% neutralization of SHIV 89.6 was tested on serum collected 2 wk after the final boost. Shown in Fig. 3, there was about a 3-fold increase in the neutralizing titer of sera from mice immunized with chimeric HA/SHIV VLPs compared with that in sera from SHIV VLP-immunized mice using both i.p. and i.n. routes ($p < 0.001$). The magnitude of neutralizing Ab production was higher in i.p. immunized animals than in i.n. immunized mice. This result shows that the enhanced immune responses induced by chimeric HA/SHIV VLPs in the absence of CD4$^+$ T cells has virus-neutralizing activity in vitro.

Intranasal immunization with chimeric HA/SHIV VLPs induces a CTL response in CD4$^+$ T cell-deficient mice

To evaluate whether chimeric HA/SHIV VLPs could enhance cellular immune responses, CTL activity against HIV Env in the immunized animal splenocytes was determined 2 wk after the final immunization. Shown in Fig. 4, splenocytes from mice immunized with HA/SHIV VLPs elicited higher percentages of HIV Env-specific lysis of target cells than those from SHIV VLP-immunized mice with both i.p. and i.n. immunizations. A high E:T cell ratio represents a high percentage of lysis of target cells. There were no substantial differences in CTL activity between i.p. and i.n. immunizations. This result indicates that incorporation of HA into SHIV VLPs could also enhance HIV Env-specific CTL immune responses in CD4$^+$ T cell-deficient mice.

Chimeric HA/SHIV VLPs efficiently bind and activate DCs

To investigate the possible mechanisms of how chimeric HA/SHIV VLPs could initiate stronger immune responses in the absence of CD4$^+$ T cell help, we examined the interaction of the VLPs with DCs. The binding capacities of SHIV VLPs and chimeric HA/SHIV VLPs to C57BL/6J BMDCs were examined and compared. As shown in Fig. 5, there was less binding of SIV-VLPs than SHIV-VLPs to the DCs, although there were ~85% DC binding to SHIV VLP binding DCs; chimeric HA/SHIV VLPs bound to 97% DCs. These data indicate that chimeric HA/SHIV VLPs have an enhanced binding activity to DCs compared with SHIV VLPs.

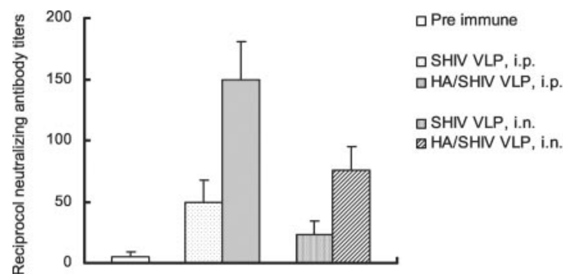

FIGURE 3. Enhanced neutralizing Ab production against SHIV 89.6 in chimeric HA/SHIV VLP-immunized CD4 KO mice. An sMAGI assay was used to detect neutralization activity of sera collected at 8 wk. Reciprocal neutralization titers to SHIV 89.6 are shown on the *y*-axis. Results are expressed as the mean 50% reduction neutralization titer from five mice per group. Error bars represent SD. Pre immune, serum from unimmunized CD4 KO mice.

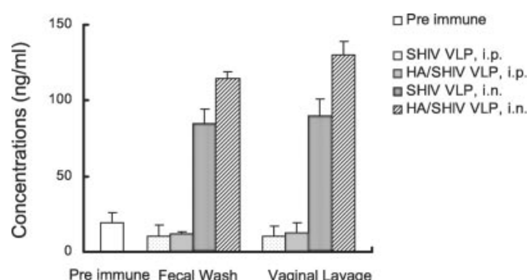

FIGURE 2. Comparison of mucosal IgA Ab responses by i.p and i.n. administrations of SHIV VLPs and chimeric HA/SHIV VLPs. Four groups of CD4$^+$ T cell-deficient mice were immunized as described above. At 8 wk, mucosal fluid fecal extract and vaginal lavage were collected. Mucosal IgA production against HIV Env was measured by quantitative ELISA. Results are expressed as the arithmetic mean ± SD from five mice per group. Pre immune, mucosal lavage collected from unimmunized CD4 KO mice.

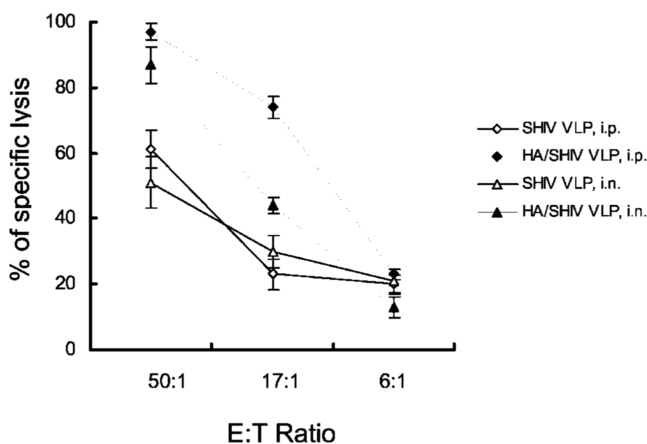

FIGURE 4. Chimeric HA/SHIV VLPs elicited an enhanced CTL activity against HIV Env. HIV Env-specific splenocyte CTL activity was determined 8 wk after the initial immunization. CTL activity against vv-HIV Env-infected EL-4 target cells was determined by using the Cytotox 96 assay kit. CTL results were obtained by pooling splenocytes from five mice per group and are representative of three independent experiments. Error bars represent the SD.

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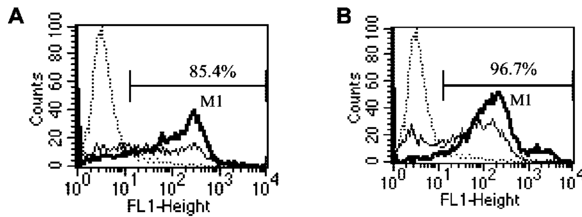

FIGURE 5. Binding of SHIV VLPs and chimeric HA/SHIV VLPs to mouse BMDCs. C57BL/6J BMDCs were incubated with SHIV VLPs or chimeric HA/SHIV VLPs for 1 h at 4°C. The primary Ab is monkey anti-SIV at a 1/500 dilution. The secondary Ab is anti-monkey conjugated with FITC at a 1/500 dilution. Cells were analyzed by FACSCalibur. Histograms shown are cell populations gated on CD11c$^+$ cells. The dotted line represents the isotype control. The fine solid line represents SIV Gag VLPs binding to DCs, and the thick solid line represents binding of SHIV VLPs (*A*) or chimeric HA/SHIV VLPs (*B*) to DCs. Binding percentages of SHIV VLPs and chimeric HA/SHIV VLPs were shown as M1 marker. The data shown represent one of three independent experiments.

To determine whether chimeric HA/SHIV VLPs could have an enhanced capacity to activate DCs, we determined the whole panel of DC activation markers, as described in *Materials and Methods*, on mouse BMDCs after different VLPs treatments. Fig. 6 shows representative DC activation markers (CD40, CD86, and MHC class II I-A^b) on CD11c$^+$ DCs treated with SHIV VLPs, chimeric HA/SHIV VLPs, LPS as a positive control and the untreated mice as a negative control. There was an ~20–40% increase in DC surface activation markers in those VLP-treated DCs compared with untreated DCs. In comparison of the activation capacity between SHIV VLPs and chimeric HA/SHIV VLPs, there was an ~10% increase in DC activation markers such as CD40, CD86, and I-A^b in chimeric HA/SHIV VLP-treated DCs compared with SHIV VLP-treated DCs. Positive control of LPS showed a strong DC activation activity. The untreated DCs showed very low levels of these DC surface activation molecules. To exclude the possible endotoxin effect on DC activation, we have also included treatment with heat-denatured VLP preparation in the DC activation assay. There was <10% increase in DC surface activation molecules by the heat-denatured VLP treatment (data not shown). These data indicated that the chimeric HA/SHIV VLPs could preferentially bind and activate DCs. Furthermore, the intact VLPs virion structure, rather than low levels of endotoxin in the VLP preparation, is important in the DC activation process.

Chimeric HA/SHIV VLPs stimulate mature DCs to secrete proinflammatory cytokine and Th1-type cytokines

To determine cytokine production profiles of chimeric HA/SHIV VLP-activated DCs, culture supernatant cytokine production was measured using the CBA kit. There was very little IL-2, IL-5, and IL-10 detected. The only detectable cytokines were TNF-α and IFN-γ, as plotted in Fig. 7. There was significantly higher TNF-α and IFN-γ production in chimeric HA/SHIV VLP-treated DCs than in SHIV VLP-treated DCs. Furthermore, chimeric HA/SHIV VLPs had a stronger activity than LPS in stimulating DCs to produce IFN-γ. These data indicate that chimeric HA/SHIV VLPs also possess a strong activation capacity to DCs and thus led to proinflammatory cytokine production as well as a key Th1-type cytokine production.

Discussion

We have demonstrated that chimeric HA/SHIV VLPs have a strong potential to stimulate both humoral and cellular immune responses to HIV Env in the absence of CD4$^+$ T cell help in CD4 KO mice. We showed enhanced IgG and IgG2a Ab production in both i.p. and i.n. immunizations with chimeric HA/SHIV VLPs. Furthermore, enhanced mucosal IgA Ab production was also found in i.n. immunization with chimeric HA/SHIV VLPs. Consistent with our previous study, chimeric HA/SHIV VLPs induced 3 times higher neutralizing Ab responses than SHIV VLPs. Furthermore, chimeric HA/SHIV VLPs could induce high CTL responses in the absence of CD4$^+$ T cell help. Additionally, we have also shown that chimeric HA/SHIV VLPs could bind and activate DC efficiently and stimulate DCs to secrete high levels of TNF-α and IFN-γ. Our study is the first to show the possible adjuvant activity of HA and indicates that the HA/SHIV VLP-activated DCs may be responsible for T and B cell activation without conventional CD4$^+$ T cell help to initiate immune responses in the CD4$^+$ T cell KO mice model.

We have previously reported that HA/SHIV VLPs can enhance immune responses in w.t. animals (C57BL/6J) (20). However, the molecular mechanisms of the effect of HA/SHIV VLPs were not investigated. The current study has been focused on the molecular mechanisms of HA/SHIV VLP-enhanced immune responses. Two

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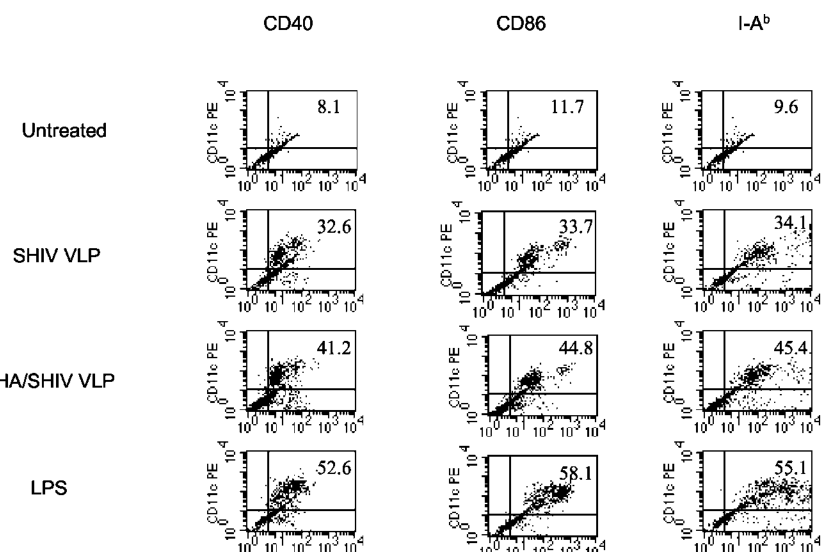

FIGURE 6. Activation of mouse BMDCs by VLPs. SHIV VLPs or chimeric HA/SHIV VLPs at a concentration of 10 μg/10^6 cells in 1 ml of PBS/1% FBS were added to DC culture medium for 2 days. As a positive control, LPS was added at 10 μg/ml. As a negative control, DCs were left untreated. Abs against DC activation surface markers conjugated with FITC or CD11c$^+$ conjugated with PE were used. FACS analysis was performed, and the results are shown as a dot plot. Shown are the representative DC activation markers (CD40, CD86, and I-A^b) presented on gated CD11c$^+$ cells. The *x*-axis represents FITC-labeled DC activation marker staining. The *y*-axis represents PE-labeled CD11c$^+$ cells. These data represent more than three experiments with DCs from different preparations.

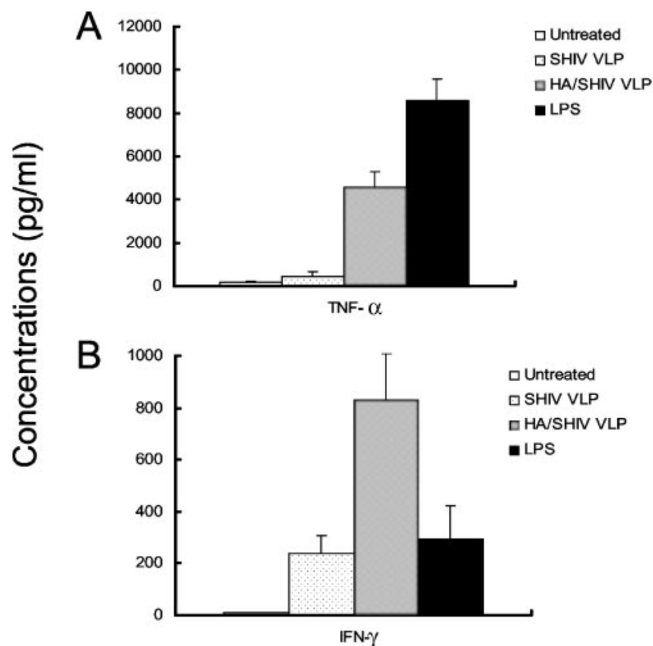

FIGURE 7. Cytokine production from DCs activated by SHIV VLPs or SHIV VLPs or chimeric HA/SHIV VLPs. Immature DCs cultured for 6 days were exposed to 10 μg/ml of either SHIV VLPs or chimeric HA/SHIV VLPs for 48 h. Immature DCs treated with LPS (10 μg/ml) were used as a positive control. Untreated DCs were used as a negative control. Culture supernatants were collected, and cytokines were detected using a commercial available CBA kit. Error bars represent the SD.

key questions have been addressed. 1). Are CD4^{+} T cells required in the effect of HA/SHIV VLPs? 2) Do HA/SHIV VLPs directly affect DC functions? In these investigations we found that HA/SHIV VLPs could enhance both cellular and humoral responses with similar patterns in w.t. and CD4 KO mice, indicating HA/SHIV VLPs could use CD4-independent pathways for inducing immune responses. This is an important discovery that could have potential applications in HIV-infected patients with low CD4 counts as a therapeutic vaccine. However, the overall magnitude of immune responses in both SHIV VLP- and HA/SHIV VLP-immunized animals was higher in w.t. mice than in CD4 KO mice, indicating that CD4 was an important pathway involving VLP-induced immune responses in general. In addition, HA/SHIV VLPs had stronger effects on DC maturation and activation than SHIV VLPs, indicating that DCs could be an important mechanism of the HA/SHIV VLP-enhanced immune response in CD4 KO mice.

Characterization of the VLPs is important in interpretation of the data. Detailed information about VLP characterization has been reported in our previous publications (19, 20, 28). Comparable amounts of Env were incorporated in both SHIV VLPs and chimeric HA/SHIV VLPs. Chimeric HA/SHIV VLPs could have changes in their biological properties compared with SHIV VLPs. However, HA incorporation may not substantially affect the VLP geometry, because electron microscopic observations did not show obvious differences in size or shape between SHIV VLPs and chimeric HA/SHIV VLPs.

CD4^{+} T cells have been considered to be the critical helper cells in inducing immune responses. It has been believed that the induction of Ab requires CD4^{+} Th cells (32). However, several recent studies have shown the induction of Ab responses in animals without CD4^{+} T cells (10, 13, 33, 34). Studies with rabies virus vaccine showed the induction of virus-neutralizing Abs to rabies that rendered partial resistance to viral challenge without CD4^{+} T cells (34). Another study with inactivated influenza virus showed IgG Ab class switch and protection from influenza live virus challenge in CD4^{+} T cell KO mice (13). In this study we have shown that IgG Ab responses can be induced by VLP immunization in CD4 KO mice, and chimeric HA/SHIV VLPs immunization can significantly increase IgG production in this animal model. Furthermore, the booster effect demonstrates that CD4 KO mice can generate memory B cells to HIV Env, especially in response to chimeric HA/SHIV VLP immunization. Influenza HA protein has a unique property of binding to sialic acid residues on the surface of many cells and has been reported to induce vigorous B cell proliferation and Ig synthesis (35, 36). Therefore, enhanced binding of HA-containing VLPs to APCs may enhance APC activation and Ag presentation, which facilitate B cell proliferation and Ab production even in the absence of CD4^{+} T cell help. Our results indicate an adjuvant effect of influenza HA incorporated into VLP particles in inducing IgG Ab production in the CD4 KO mouse model. However, the magnitude of IgG Ab induction in CD4 KO mice was still lower than that in w.t. C57BL/6J mice using the same immunization procedures. This may indicate that the specific signals from alternative molecules or cells that activate B cells are not as strong as the signals from CD4^{+} T cells. In this study HA protein incorporated on the surface of the VLPs could be the important molecule that binds and activates APCs, which facilitate Ab production from B cells in the absence of CD4^{+} T cells. HA/SHIV VLPs could also directly affect B cell functions. Although what signals determine Th1- or Th2-type immune responses are still not known, we have reported that SHIV VLPs could induce mixed types of Th1/Th2 immune responses in w.t. mice (20). We again found in this study that chimeric HA/SHIV VLPs could induce high levels of IgG1 as well as IgG2a in CD4 KO mice, indicating a capacity to induce mixed subtypes of IgG Ab responses in CD4 KO mice. Usually, humoral immune responses are mainly observed in a natural influenza infection (37). Thus, HA binding to the cell surface receptor may deliver signals that facilitate immune responses similar to those observed in influenza infection.

As for the induction of mucosal IgA production, chimeric HA/SHIV VLPs may have an enhanced binding of VLPs with mucosal surface cells, which may be a mechanism for enhanced mucosal Ab production in the absence of CD4^{+} T cells. Although a role for DC-B cell interactions in the generation of IgA responses on the mucosal surface is not yet determined, it might be relevant that chimeric HA/SHIV VLP-treated DCs remain active and express VLPs surface Ags on the cell membrane for a prolonged period. Such a strong Ag presentation pattern may provide the basis for cognate DC interactions with HIV Env-specific B cells as well as deliver a potent stimulatory signal to B cells via BCR cross-linking. This idea is consistent with evidence that the early IgA response in local lymph nodes after influenza infection is dependent on virus replication (38) and is directed exclusively against virion envelope gp (39). HIV-specific neutralizing Ab production is considered to be one of the important aspects in HIV vaccine development (30–43). One of the important findings in this study is the neutralizing Ab production capability of CD4 KO mice in response to chimeric HA/SHIV VLP immunization. With both i.p. and i.n. routes, chimeric HA/SHIV VLPs consistently induce ~3-fold higher neutralizing Ab than SHIV VLPs in the absence of Th cells.

DCs and macrophages have been shown to be involved in the processing of VLPs (16, 23, 44, 45). Recent studies have emphasized the central role of DCs in initiating adaptive immune responses after exposure to various pathogens, such as pathogen-associated molecular patterns in their microenvironment (46). We have shown in this study the enhanced binding of HA/SHIV VLPs with DCs and the preferential activation of DCs by chimeric HA/

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SHIV VLPs. Interestingly, we have found that three different kinds of VLPs can bind to DCs at different binding efficiencies. Chimeric HA/SHIV VLPs have the highest binding capacity, and SIV VLPs have the lowest binding capacity. There could be many factors contributing to the enhanced binding of chimeric HA/VLPs or SHIV VLPs to the DCs compared with SIV VLPs. The Env incorporated on the SHIV VLPs could contribute to the binding of VLPs to DCs, which have high DC-specific ICAM-3 grabbing nonintegrin expression on the surface. In addition, the HA incorporated on the HA/SHIV VLPs could contribute to binding to the sialic acid-containing residues on the DC surface. The differences in their binding capacity to DCs could lead to differences in DC Ag-processing functions, resulting in different magnitudes of immune responses. Our data suggest that the HA-binding properties of chimeric HA/SHIV VLPs favor a quick uptake and processing of these VLPs by APCs. Furthermore, because of the fusion property of HA, chimeric HA/SHIV VLPs may gain access to the cytosol after fusion between lipidic structure and cellular membrane, as shown for liposomes (47, 48). The high immunogenicity of HA/SHIV VLPs could also be due to their particulate form, which can favor their optimal delivery to the class I Ag presentation pathway like other VLPs (49, 50). We suggest that, like CFA, which allows the induction of Th cell-independent CTL responses to OVA, particulate chimeric HA/SHIV VLPs could activate DCs directly, circumventing their need for activation through Th cells.

We have shown that immunization with HA/SHIV VLPs can enhance the CTL response in CD4 KO mice. Several hypothesis may explain the strong potential of chimeric HA/SHIV VLP to induce the $CD8^+$ CTL response in the absence of $CD4^+$ T cells. $CD8^+$ CTLs are responsible for the lysis of Ag-bearing target cells. Generation of effective CTL responses often requires help from Th cells (4, 15, 51, 52). The current model for CD8 T cell activation involves the concept of licensing of APCs (53–55). Although the requirements for the activation step are not fully elucidated, several studies suggested that CD40, B7-1, B7-2, and IL-12 signaling may play roles (26, 56–59). CD40-CD40L interaction between APCs and CD4 T cells results in up-regulation of costimulatory molecules such as CD80/CD86 levels and other factors on APCs and licenses APCs to subsequently interact with and activate CD8 T cells, thereby bypassing the CD4 T cell requirement (60, 61). HA appears to replace the licensing function of $CD4^+$ T cells, presumably by initiating similar downstream signaling events in APCs as suggested by other studies with CpG-gp120 immunization (62, 63). This hypothesis requires both HA and HIV Env colocalized to the same APCs. They may require modifying APCs and converting them into stimulatory cells for CTL priming (5). The activation of CTL in the absence of T cell help strongly suggests that HA may replace the licensing functions of the Th-APC interaction. Current data indicate that chimeric HA/SHIV VLPs stimulation can up-regulate a number of proinflammatory cytokines and surface molecules, which may account for this effect. Investigation into the roles of these molecules in direct activation of $CD8^+$ T cells and B cells may provide mechanistic insight into this phenomenon.

We report in this study that in the absence of Th cells, exogenous chimeric HA/SHIV VLPs could bind and activate DCs and initiate both humoral and cellular immune responses. Cytokines TNF-α and IFN-γ derived from activated DCs could mediate the durable vaccine immunity. The HA molecule incorporated on the surface of chimeric VLPs could play an important role in activating immune system. The activated memory $CD8^+$ cells in a MHC class I-restricted manner and $CD8^+$ T cells could also rely on alternative mechanisms for the robust vaccine immunity in the absence of some of these factors. Our results demonstrate an interesting plasticity of immunity in compromised hosts at both cellular and molecular levels and point to the feasibility of developing vaccines against HIV infections in patients with few or no $CD4^+$ T cells. Based on these results, chimeric HA/SHIV VLP-based immunization holds great promise for the development of preventive and therapeutic HIV vaccines.

References

1. Bachmann, M. F., and R. M. Zinkernagel. 1996. The influence of virus structure on antibody responses and virus serotype formation. *Immunol. Today 17:553*.
2. Oxenius, A., R. M. Zinkernagel, and H. Hengartner. 1998. $CD4^+$ T-cell induction and effector functions: a comparison of immunity against soluble antigens and viral infections. *Adv. Immunol. 70:313*.
3. Parker, D. C. 1993. T cell-dependent B cell activation. *Annu. Rev. Immunol. 11:331*.
4. Keene, J. A., and J. Forman. 1982. Helper activity is required for the in vivo generation of cytotoxic T lymphocytes. *J. Exp. Med. 155:768*.
5. Guerder, S., and P. Matzinger. 1992. A fail-safe mechanism for maintaining self-tolerance. *J. Exp. Med. 176:553*.
6. Buller, R. M., K. L. Holmes, A. Hugin, T. N. Frederickson, and H. C. Morse III. 1987. Induction of cytotoxic T-cell responses in vivo in the absence of CD4 helper cells. *Nature 328:77*.
7. Ahmed, R., L. D. Butler, and L. Bhatti. 1988. $T4^+$ T helper cell function in vivo: differential requirement for induction of antiviral cytotoxic T-cell and antibody responses. *J. Virol. 62:2102*.
8. Szomolanyi-Tsuda, E., Q. P. Le, R. L. Garcea, and R. M. Welsh. 1998. T-cell-independent immunoglobulin G responses in vivo are elicited by live-virus infection but not by immunization with viral proteins or virus-like particles. *J. Virol. 72:6665*.
9. Szomolanyi-Tsuda, E., and R. M. Welsh. 1998. T-cell-independent antiviral antibody responses. *Curr. Opin. Immunol. 10:431*.
10. Bachmann, M. F., H. Hengartner, and R. M. Zinkernagel. 1995. T helper cell-independent neutralizing B cell response against vesicular stomatitis virus: role of antigen patterns in B cell induction? *Eur. J. Immunol. 25:3445*.
11. Bachmann, M. F., C. Bast, H. Hengartner, and R. M. Zinkernagel. 1994. Immunogenicity of a viral model vaccine after different inactivation procedures. *Med Microbiol. Immunol. 183:95*.
12. Bachmann, M. F., T. M. Kundig, C. P. Kalberer, H. Hengartner, and R. M. Zinkernagel. 1993. Formalin inactivation of vesicular stomatitis virus impairs T-cell- but not T-help-independent B-cell responses. *J. Virol. 67:3917*.
13. Sha, Z., and R. W. Compans. 2000. Induction of $CD4^+$ T-cell-independent immunoglobulin responses by inactivated influenza virus. *J. Virol. 74:4999*.
14. Moron, G., P. Rueda, I. Casal, and C. Leclerc. 2002. $CD8\alpha^-$ $CD11b^+$ dendritic cells present exogenous virus-like particles to $CD8^+$ T cells and subsequently express $CD8\alpha$ and CD205 molecules. *J. Exp. Med. 195:1233*.
15. Bennett, S. R., F. R. Carbone, F. Karamalis, J. F. Miller, and W. R. Heath. 1997. Induction of a $CD8^+$ cytotoxic T lymphocyte response by cross-priming requires cognate $CD4^+$ T cell help. *J. Exp. Med. 186:65*.
16. Bohm, W., R. Schirmbeck, A. Elbe, K. Melber, D. Diminky, G. Kraal, N. van Rooijen, Y. Barenholz, and J. Reimann. 1995. Exogenous hepatitis B surface antigen particles processed by dendritic cells or macrophages prime murine MHC class I-restricted cytotoxic T lymphocytes in vivo. *J. Immunol. 155:3313*.
17. Oliveira-Ferreira, J., Y. Myahira, G. T. Layton, N. Savage, M. Esteban, D. Rodriguez, J. R. Rodriguez, R. S. Nussenzweig, and F. Zavala. 2000. Immunogenicity of Ty-VLP bearing a $CD8^+$ T cell epitope of the CS protein of *P. yoelii*: enhanced memory response by boosting with recombinant vaccinia virus. *Vaccine 18:2923*.
18. Chackerian, B., D. R. Lowy, and J. T. Schiller. 2001. Conjugation of a self-antigen to papillomavirus-like particles allows for efficient induction of protective autoantibodies. *J. Clin. Invest. 108:415*.
19. Yao, Q., V. Vuong, M. Li, and R. W. Compans. 2002. Intranasal immunization with SIV virus-like particles (VLPs) elicits systemic and mucosal immunity. *Vaccine 20:2537*.
20. Guo, L., X. Lu, S. M. Kang, C. Chen, R. W. Compans, and Q. Yao. 2003. Enhancement of mucosal immune responses by chimeric influenza HA/SHIV virus-like particles. *Virology 313:502*.
21. Allsopp, C. E., M. Plebanski, S. Gilbert, R. E. Sinden, S. Harris, G. Frankel, G. Dougan, C. Hioe, D. Nixon, E. Paoletti, et al. 1996. Comparison of numerous delivery systems for the induction of cytotoxic T lymphocytes by immunization. *Eur. J. Immunol. 26:1951*.
22. Sedlik, C., M. Saron, J. Sarraseca, I. Casal, and C. Leclerc. 1997. Recombinant parvovirus-like particles as an antigen carrier: a novel nonreplicative exogenous antigen to elicit protective antiviral cytotoxic T cells. *Proc. Natl. Acad. Sci. USA 94:7503*.
23. Rudolf, M. P., S. C. Fausch, D. M. Da Silva, and W. M. Kast. 2001. Human dendritic cells are activated by chimeric human papillomavirus type-16 virus-like particles and induce epitope-specific human T cell responses in vitro. *J. Immunol. 166:5917*.
24. Ball, J. M., D. Y. Graham, A. R. Opekun, M. A. Gilger, R. A. Guerrero, and M. K. Estes. 1999. Recombinant Norwalk virus-like particles given orally to volunteers: phase I study. *Gastroenterology 117:40*.
25. Horner, A. A., S. K. Datta, K. Takabayashi, I. M. Belyakov, T. Hayashi, N. Cinman, M. D. Nguyen, J. H. Van Uden, J. A. Berzofsky, D. D. Richman, et

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al. 2001. Immunostimulatory DNA-based vaccines elicit multifaceted immune responses against HIV at systemic and mucosal sites. *J. Immunol. 167:1584*.
26. Cho, H. J., K. Takabayashi, P. M. Cheng, M. D. Nguyen, M. Corr, S. Tuck, and E. Raz. 2000. Immunostimulatory DNA-based vaccines induce cytotoxic lymphocyte activity by a T-helper cell-independent mechanism. *Nat. Biotechnol. 18:509*.
27. Hallenberger, S., S. P. Tucker, R. J. Owens, H. B. Bernstein, and R. W. Compans. 1993. Secretion of a truncated form of the human immunodeficiency virus type 1 envelope glycoprotein. *Virology 193:510*.
28. Yao, Q., F. M. Kuhlmann, R. Eller, R. W. Compans, and C. Chen. 2000. Production and characterization of simian–human immunodeficiency virus-like particles. *AIDS Res. Hum. Retroviruses 16:227*.
29. Rahemtulla, A., W. P. Fung-Leung, M. W. Schilham, T. M. Kundig, S. R. Sambhara, A. Narendran, A. Arabian, A. Wakeham, C. J. Paige, R. M. Zinkernagel, et al. 1991. Normal development and function of CD8$^+$ cells but markedly decreased helper cell activity in mice lacking CD4. *Nature 353:180*.
30. Chackerian, B., N. L. Haigwood, and J. Overbaugh. 1995. Characterization of a CD4-expressing macaque cell line that can detect virus after a single replication cycle and can be infected by diverse simian immunodeficiency virus isolates. *Virology 213:386*.
31. Wang, H. Y., T. Fu, G. Wang, G. Zeng, D. M. Perry-Lalley, J. C. Yang, N. P. Restifo, P. Hwu, and R. F. Wang. 2002. Induction of CD4$^+$ T cell-dependent antitumor immunity by TAT-mediated tumor antigen delivery into dendritic cells. *J. Clin. Invest. 109:1463*.
32. Noelle, R. J., J. McCann, L. Marshall, and W. C. Bartlett. 1989. Cognate interactions between helper T cells and B cells. III. Contact-dependent, lymphokine-independent induction of B cell cycle entry by activated helper T cells. *J. Immunol. 143:1807*.
33. Szomolanyi-Tsuda, E., J. D. Brien, J. E. Dorgan, R. L. Garcea, R. T. Woodland, and R. M. Welsh. 2001. Antiviral T-cell-independent type 2 antibody responses induced in vivo in the absence of T and NK cells. *Virology 280:160*.
34. Xiang, Z. Q., B. B. Knowles, J. W. McCarrick, and H. C. Ertl. 1995. Immune effector mechanisms required for protection to rabies virus. *Virology 214:398*.
35. Rott, O., J. Charreire, and E. Cash. 1996. Influenza A virus hemagglutinin is a B cell-superstimulatory lectin. *Med. Microbiol. Immunol. 184:185*.
36. Rott, O., J. Charreire, K. Mignon-Godefroy, and E. Cash. 1995. B cell superstimulatory influenza virus activates peritoneal B cells. *J. Immunol. 155:134*.
37. Grunewald, S. M., C. Hahn, G. Wohlleben, M. Teufel, T. Major, H. Moll, E. B. Brocker, and K. J. Erb. 2002. Infection with influenza a virus leads to flu antigen-induced cutaneous anaphylaxis in mice. *J. Invest. Dermatol. 118:645*.
38. Marshall, D., R. Sealy, M. Sangster, and C. Coleclough. 1999. TH cells primed during influenza virus infection provide help for qualitatively distinct antibody responses to subsequent immunization. *J. Immunol. 163:4673*.
39. Sealy, R., S. Surman, J. L. Hurwitz, and C. Coleclough. 2003. Antibody response to influenza infection of mice: different patterns for glycoprotein and nucleocapsid antigens. *Immunology 108:431*.
40. Mascola, J. R., M. G. Lewis, G. Stiegler, D. Harris, T. C. VanCott, D. Hayes, M. K. Louder, C. R. Brown, C. V. Sapan, S. S. Frankel, et al. 1999. Protection of macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies. *J. Virol. 73:4009*.
41. Shibata, R., T. Igarashi, N. Haigwood, A. Buckler-White, R. Ogert, W. Ross, R. Willey, M. W. Cho, and M. A. Martin. 1999. Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV chimeric virus infections of macaque monkeys. *Nat. Med. 5:204*.
42. Mascola, J. R., G. Stiegler, T. C. VanCott, H. Katinger, C. B. Carpenter, C. E. Hanson, H. Beary, D. Hayes, S. S. Frankel, D. L. Birx, et al. 2000. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. *Nat. Med. 6:207*.
43. Parren, P. W., P. A. Marx, A. J. Hessell, A. Luckay, J. Harouse, C. Cheng-Mayer, J. P. Moore, and D. R. Burton. 2001. Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization in vitro. *J. Virol. 75:8340*.
44. Bachmann, M. F., M. B. Lutz, G. T. Layton, S. J. Harris, T. Fehr, M. Rescigno, and P. Ricciardi-Castagnoli. 1996. Dendritic cells process exogenous viral proteins and virus-like particles for class I presentation to CD8$^+$ cytotoxic T lymphocytes. *Eur. J. Immunol. 26:2595*.
45. Lenz, P., P. M. Day, Y. Y. Pang, S. A. Frye, P. N. Jensen, D. R. Lowy, and J. T. Schiller. 2001. Papillomavirus-like particles induce acute activation of dendritic cells. *J. Immunol. 166:5346*.
46. Gallucci, S., M. Lolkema, and P. Matzinger. 1999. Natural adjuvants: endogenous activators of dendritic cells. *Nat. Med. 5:1249*.
47. Zhou, F., B. T. Rouse, and L. Huang. 1992. Induction of cytotoxic T lymphocytes in vivo with protein antigen entrapped in membranous vehicles. *J. Immunol. 149:1599*.
48. Nair, S., A. M. Buiting, R. J. Rouse, N. Van Rooijen, L. Huang, and B. T. Rouse. 1995. Role of macrophages and dendritic cells in primary cytotoxic T lymphocyte responses. *Int. Immunol. 7:679*.
49. Schirmbeck, R., K. Melber, and J. Reimann. 1995. Hepatitis B virus small surface antigen particles are processed in a novel endosomal pathway for major histocompatibility complex class I-restricted epitope presentation. *Eur. J. Immunol. 25:1063*.
50. Schirmbeck, R., W. Bohm, K. Melber, and J. Reimann. 1995. Processing of exogenous heat-aggregated (denatured) and particulate (native) hepatitis B surface antigen for class I-restricted epitope presentation. *J. Immunol. 155:4676*.
51. von Herrath, M. G., M. Yokoyama, J. Dockter, M. B. Oldstone, and J. L. Whitton. 1996. CD4-deficient mice have reduced levels of memory cytotoxic T lymphocytes after immunization and show diminished resistance to subsequent virus challenge. *J. Virol. 70:1072*.
52. Cardin, R. D., J. W. Brooks, S. R. Sarawar, and P. C. Doherty. 1996. Progressive loss of CD8$^+$ T cell-mediated control of a γ-herpesvirus in the absence of CD4$^+$ T cells. *J. Exp. Med. 184:863*.
53. Bennett, S. R., F. R. Carbone, F. Karamalis, R. A. Flavell, J. F. Miller, and W. R. Heath. 1998. Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature 393:478*.
54. Ridge, J. P., F. Di Rosa, and P. Matzinger. 1998. A conditioned dendritic cell can be a temporal bridge between a CD4$^+$ T-helper and a T-killer cell. *Nature 393:474*.
55. Schoenberger, S. P., R. E. Toes, E. I. van der Voort, R. Offringa, and C. J. Melief. 1998. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature 393:480*.
56. Harding, F. A., and J. P. Allison. 1993. CD28–B7 interactions allow the induction of CD8$^+$ cytotoxic T lymphocytes in the absence of exogenous help. *J. Exp. Med. 177:1791*.
57. Grewal, I. S., and R. A. Flavell. 1996. The role of CD40 ligand in costimulation and T-cell activation. *Immunol. Rev. 153:85*.
58. Sigal, L. J., H. Reiser, and K. L. Rock. 1998. The role of B7-1 and B7-2 costimulation for the generation of CTL responses in vivo. *J. Immunol. 161:2740*.
59. Trinchieri, G. 1995. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu. Rev. Immunol. 13:251*.
60. Cella, M., D. Scheidegger, K. Palmer-Lehmann, P. Lane, A. Lanzavecchia, and G. Alber. 1996. Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J. Exp. Med. 184:747*.
61. Koch, F., U. Stanzl, P. Jennewein, K. Janke, C. Heufler, E. Kampgen, N. Romani, and G. Schuler. 1996. High level IL-12 production by murine dendritic cells: upregulation via MHC class II and CD40 molecules and downregulation by IL-4 and IL-10. *J. Exp. Med. 184:741*.
62. Hacker, H., H. Mischak, T. Miethke, S. Liptay, R. Schmid, T. Sparwasser, K. Heeg, G. B. Lipford, and H. Wagner. 1998. CpG-DNA-specific activation of antigen-presenting cells requires stress kinase activity and is preceded by non-specific endocytosis and endosomal maturation. *EMBO J. 17:6230*.
63. Hacker, H., R. M. Vabulas, O. Takeuchi, K. Hoshino, S. Akira, and H. Wagner. 2000. Immune cell activation by bacterial CpG-DNA through myeloid differentiation marker 88 and tumor necrosis factor receptor-associated factor (TRAF)6. *J. Exp. Med. 192:595*.

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