CD8α−CD11b+ Dendritic Cells Present Exogenous Virus-like Particles to CD8+ T Cells and Subsequently Express CD8α and CD205 Molecules

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Abstract

Recombinant porcine parvovirus virus-like particles (PPV-VLPs) are particulate exogenous antigens that induce a strong, specific cytotoxic T lymphocyte (CTL) response in the absence of adjuvant. In the present report, we demonstrate in vivo that dendritic cells (DCs) present PPV-VLPs to CD8+ T cells after intracellular processing. PPV-VLPs are captured by DCs with a high efficiency, which results in the delivery of these exogenous antigens to 50% of the whole spleen DC population. In vivo, a few hours after injection, PPV-VLPs are presented exclusively to CD8+ T cells by CD8α− DCs, whereas 15 hours later they are presented mainly by CD8α+ DCs. After PPV-VLPs processing, a fraction of CD11b+ DCs undergo phenotypic changes, i.e., the up-regulation of CD8α and CD205 and the loss of CD4 molecules on their surface. The failure to detect mRNA coding for CD8α in CD11b+ DCs suggests that CD8α expression by these cells is not due to de novo synthesis. In recombination-activating gene knockout mice (Rag−/−), CD11b+ DCs did not express CD8α and PPV-VLPs presentation by CD8α+ DCs was severely diminished. These results indicate that both CD8α− and CD8α+ DCs play an important role in the induction of CTL responses by exogenous antigens, such as VLP.

Key words: virus-like particles • dendritic cells • cross-priming • CTL • exogenous antigens

Introduction

The induction of CTL responses requires the presentation of antigen-derived peptides associated to MHC class I molecules on the surface of APCs to specific CD8+ T cells. These peptides essentially derive from antigens processed in the cytosol of APCs. Thus, antigens that do not reach the cytosol of APCs should not elicit a CTL response. However, it is now well established that host APCs can process exogenous cell-associated antigens and present them associated to MHC class I molecules through a process called cross-priming (1, 2). In this process, cell-associated antigens gain access to the MHC class I pathway by the transfer of these antigens from cells that expressed or carried them to APCs. Soluble exogenous antigens can also gain access to the cytosol of APCs through an alternative pathway exclusive for macrophage and dendritic cells (DCs)* (3), although cell-associated antigens are much more efficiently presented than soluble antigens (4, 5). Both macrophages (6) and DCs (7) have been reported to cross-present antigens, but only DCs are able to stimulate naive CD8+ T cells (8). Two routes of cross-priming have been proposed. One route involves the passage of antigens from endosomes to cytosol and in the other route the antigens do not escape from endosomes and are processed inside these vesicles (9). The first route seems to be mostly used by DCs whereas the second one is specific to macrophages (10).

DCs do not constitute a homogeneous cell population. On the basis of the expression of CD8α homodimer and CD4 molecules, these three subpopulations of DCs have been described in murine spleen: CD4+CD8α−, CD4+CD8α− (both are CD11b+), and CD8α+CD11c+ (CD11blow) (11, 12). However, so far the attention has

*Abbreviations used in this paper: B2M, β2-microglobulin; DC, dendritic cell; LCMV, lymphocytic choriomeningitis virus; MACS, magnetic-activated cell sorting; OVA257–264, peptide corresponding to the sequence of amino acids 257–264 of the chicken egg albumin; PPV, porcine parvovirus; PPV-VLPs-OVA, PPV virus-like particles carrying OVA257–264; Rag, recombination-activating gene; RT, reverse transcription; TAP, transporter associated with antigen processing; VLP, virus-like particle; VP, viral protein.

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been focused mainly on the study of CD8α− and CD8α+ DCs, which until recently have been considered to be derived from myeloid and lymphoid progenitors, respectively (13, 14). It has recently been reported that CD8α+ but not CD8α− DCs can cross-prime CD8+ T cells in vivo excluding a role for CD8α− DCs in CTL induction (15).

Virus-like particles (VLPs) have clearly revealed an exceptional capacity to trigger CTL responses (3, 16–19). However, the mechanisms of uptake, processing, and presentation of these exogenous particles remain unclear. In particular, the APC involved in the induction of CTL response by VLPs is still unknown. Indeed, DCs and macrophages have been shown to be involved in the processing of VLPs (16, 20–22), but no direct in vivo evidence has been obtained identifying the APC that can uptake, process, and present VLPs.

We have developed an antigen delivery system based on nonreplicative, recombinant porcine parvovirus (PPV)-VLPs formed by the self-assembly of the viral protein (VP)2 of PPV (19, 23). The VP2 protein, the most abundant structural VP of the PPV capsid (24) and carrying foreign CD8+ T cell epitopes, self-assembles into 25–nm pseudo viral particles after expression in insect cells (19). Mice immunized with these PPV-VLPs, carrying a CD8+ T cell epitope from the lymphocytic choriomeningitis virus (LCMV) nucleoprotein and in the absence of adjuvant, developed a CTL response against LCMV that protected mice against a lethal intracerebral injection of LCMV based (LCMV) nucleoprotein and in the absence of adjuvant, deriving from myeloid and lymphoid progenitors, respectively (24) and carrying foreign structural VP of the PPV capsid (19, 25). This cytotoxic response was restricted to MHC class I molecules and mediated by CD8+ T cells (19).

We have studied the mechanisms of in vivo presentation of particulate exogenous antigens using PPV-VLPs as a model to determine whether particulate antigens can be captured and processed directly by DCs or if they induce CTL response by cross-priming after capture by other cells. In this report, we demonstrate that PPV-VLPs target DCs with a very high efficiency and directly induce a specific CTL response without cross-priming. CD8α− and CD8α+ DCs capture and process these particles. We also establish that CD8α− DCs play an important role in CTL induction by these exogenous antigens. Furthermore, this study demonstrates for the first time that stimulation by VLPs induces phenotypic changes on CD8α− DCs, which leads to the acquisition of several surface molecules and the loss of others.

**Materials and Methods**

*Mice.* 6–8-wk-old female C57BL/6 (H-2b) mice were purchased from Janvier. Transporter associated with antigen processing (TAP)− female knockout mice (TAP−/−) were a gift from A. Bandeira (Institut Pasteur, Paris, France). Recombination-activating gene (Rag2) knockout mice (Rag2−/−) and β2-microglobulin (B2M−/−) knockout mice were obtained from the Centre de Développement des Techniques Avancées pour l’Expérimentation Animale (Orléans, France). All animals were bred on a pathogen-free background. The mice were maintained under specific pathogen-free conditions.

**PPV-VLPs.** The construction, characterization, and purification of recombinant and control PPV-VLPs were previously described in detail (19). In brief, the VP2 gene was expressed with the 257–264 peptide plus natural flanking sequences (LEQLESI-INFEEKL) from chicken egg ovalbumin (OVA257–264) in its 5′ end (PPV-VLPs carrying the OVA257–264 epitope [PPV-VLPs-OVA]) or without this sequence (PPV-VLPs) using a baculovirus vector system. After the infection of Sf9 insect cells, the recombinant VLPs were purified by salt precipitation with 20% ammonium sulfate followed by dialysis. Characterization of PPV-VLPs-OVA and PPV-VLPs by CsCl sedimentation analysis and electron microscopy revealed identical properties to those of native PPV virions. In some experiments, PPV-VLPs-OVA were labeled with the fluorochrome Alexa 488, using the Alexa Fluor™ 488 Protein Labeling Kit (Molecular Probes) according to the manufacturer’s instructions.

The concentration of PPV-VLPs-OVA was determined by densitometry and by double-antibody sandwich ELISA. The densitometric assay was performed with 1D Image Analysis Software 2.0.1 (Eastman Kodak Co.) using BSA as reference. The double-antibody sandwich ELISA was performed as previously described (26), using as capture antibody the anti-PPV mAb 15C5 and as detection antibody the anti-PPV biotinylated mAb 13C6 (27). Highly purified PPV-VLPs from size exclusion chromatography were used as standard reference. PPV-VLPs are 25–nm particles formed by 60 copies of VP2 (64 kD), and therefore the molecular mass of the particles is 3,840 kD.

Endotoxin values were determined in each sample of VLPs, using the Limulus amebocyte lysate test (BioWhittaker Inc.). For PPV-VLPs, endotoxin values were <500 pg/mg of protein and for PPV-VLPs-OVA, <10 ng/mg.

**Peptides and Cell Lines.** The OVA257–264 peptide (SIINFEKL) that corresponds to an immunodominant H-2b-restricted CTL epitope of OVA was purchased from NeoSystem. B3Z, a CD8+ T cell hybridoma (28), specific for OVA257–264 epitope in the context of Kb, was a gift from N. Shastri (University of California, Berkeley, CA). The thymoma EL-4 was obtained from American Type Culture Collection.

**Preparation of DCs and Other APCs.** Spleens from either normal or immunized mice were removed and treated for 45 min at 37°C with 400 U/ml collagenase type IV and 50 μg/ml DNase I (Boehringer) in RPMI 1640. After inhibition of collagenase activity with 6 mM EDTA in PBS, spleens were dissociated in Ca2+- and Mg2+-free PBS in the presence of 2.5 mM EDTA and 0.5% FCS (GIBCO BRL) for in vitro and ex vivo assays or BSA (Sigma-Aldrich) for immunization with DCs. In all assays involving DCs, the same lot of endotoxin-free FCS (as determined by Limulus amebocyte lysate test) was used (batch 30132605). All solutions were also tested for endotoxin-free FCS (as determined by Limulus amebocyte lysate test) was used (batch 30132605). All solutions were also tested for endotoxin-free FCS (as determined by Limulus amebocyte lysate test) was used (batch 30132605) and coloidal super-paramagnetic microbeads, conjugated to anti-CD11c mAb (magnetic-activated cell sorting [MACS]-anti-CD11c, N418 clone; Miltenyi Biotec), according to the manufacturer’s instructions. CD11c+ cells were positively selected with high speed MACS (AutoMACS; Miltenyi Biotec). The purified DC preparations contained 3–10% autofluorescent cells (defined as double positive cells in a FL2 vs. FL3 dot plot without antibody labeling). The purity of DC preparations (excluding autofluorescent cells) was always 95–99%. CD11c+ cells were H-2 Kb+, I-Ab low, CD4low, CD8bom, and CD86−. 25–30% were CD8α+ and 60–70% were CD8α−CD11b+.

All spleen cell suspensions were prepared and blocked with anti-CD16/32 (2.4G2 clone; BD PharMingen) and with colloidal super-paramagnetic microbeads, conjugated to anti-CD11c mAb (magnetic-activated cell sorting [MACS]-anti-CD11c, N418 clone; Miltenyi Biotec), according to the manufacturer’s instructions. CD11c+ cells were positively selected with high speed MACS (AutoMACS; Miltenyi Biotec). The purified DC preparations contained 3–10% autofluorescent cells (defined as double positive cells in a FL2 vs. FL3 dot plot without antibody labeling). The purity of DC preparations (excluding autofluorescent cells) was always 95–99%. CD11c+ cells were H-2 Kb+, I-Ab low, CD4low, CD8bom, and CD86−. 25–30% were CD8α+ and 60–70% were CD8α−CD11b+. B220+ spleen cells were obtained according to the same pro-
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and propidium iodide was added to label dead cells. A minimum block unspecific binding of primary antibody and then stained control of specificity.

EL-4 cells. Spontaneous release was obtained with target cells in-

Maximum release was determined by adding 1% Triton X-100 to

tor cells was tested on 51Cr-labeled EL-4 target cells pulsed with

OVA, PPV-VLPs, or OVA257–264 peptide) for 4 h in 96-well cul-

ture microplates in a final volume of 0.2 ml of RPMI 1640 Glutamax-I, plus 5 M 2-ME, 100 IU/ml penicillin, 100 µg/ml streptomycin, and 10% FCS (10% RPMI; all from GibCO BRL). Subsequently, APCs were washed with 10% RPMI and incubated overnight with 10⁵ B3Z cells/well in a final volume of 0.2 ml 10% RPMI at 37°C. The stimulation of B3Z cells was monitored by IL-2 release in supernatants, which was measured using the classic CTLL-2 bioassay. 10⁴ cells/well of the CTLL-2 cell line were cultured with 100-µl supernatant in a final volume of 0.2 ml 2-ME, 100 IU/ml penicillin, 100 µg/ml streptomycin, and 10% FCS (10% RPMI; all from GibCO BRL). Incorporation of thymidine was detected by cell scintillation counting. All experiments were done in duplicate. Results are expressed in counts per minute.

For ex vivo assays, PPV-VLPs-OVA or PPV-VLPs were in-

jected into the retro-orbital venous sinus of mice. APCs were iso-

lated and incubated with B3Z hybridoma overnight in the same conditions as previously described.

CD11b⁺CD8⁺ spleen cells (10⁶ cells/well) were first pulsed with antigen (PPV-VLPs-OVA, PPV-VLPs, or OVA257–264 peptide) for 4 h in 96-well culture microplates in a final volume of 0.2 ml of RPMI 1640 Glutamax-I, plus 5 µg/ml streptomycin, 100 µg/ml penicillin, 100 µg/ml streptomycin, and 10% FCS (100 µg/ml streptomycin, and 10% FCS (10% RPMI; all from GibCO BRL). Subsequently, APCs were washed with 10% RPMI and incubated overnight with 10⁵ B3Z cells/well in a final volume of 0.2 ml 10% RPMI at 37°C. The stimulation of B3Z cells was monitored by IL-2 release in supernatants, which was measured using the classic CTLL-2 bioassay. 10⁴ cells/well of the CTLL-2 cell line were cultured with 100-µl supernatant in a final volume of 0.2 ml 2-ME, 100 IU/ml penicillin, 100 µg/ml streptomycin, and 10% FCS (10% RPMI; all from GibCO BRL). Incorporation of thymidine was detected by cell scintillation counting. All experiments were done in duplicate. Results are expressed in counts per minute.

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lated and incubated with B3Z hybridoma overnight in the same conditions as previously described.

Flow Cytometry. Cells were preincubated with a rat anti-

CD16/32 mAb (2.4G2 clone; BD PharmMingen) for 15 min to block unspecific binding of primary antibody and then stained with the primary antibodies for 30 min. Cells were washed twice and propidium iodide was added to label dead cells. A minimum of 2 × 10⁴ events were acquired for each sample on FACScan™ or FACSCalibur® cytometers and analyzed using CELLQuest™ software (all from BD Biosciences). The following mAbs were used: anti-CD3e (145-2C11 clone), anti-CD4 (L3T4, RM4-5 clone), anti-CD8α (Ly-2, 53-6.7 clone), anti-CD8β (Ly-3.2, 53-5.8 clone), anti-CD11b (Mac-1α, M1/70 clone), anti-CD11c (HL-3 clone), anti-CD45R (B220, RA3-6B2 clone), anti-CD86 (B7.2, GL1 clone), and CD90 (Thy1.2, 30-H12), all purchased from BD PharmMingen. Anti-CD86 (CT-CD86 clone; Caltag) and anti-CD205 (NLD-145 clone; Cedarlane Laboratories Ltd.) were also used.

Reverse Transcription (RT)-PCR. Total RNA was extracted using RNA Plus solution (Quantum Appignane Société Anonyme), from 5–10 × 10⁵ purified DCs subpopulations (before or after overnight culture). cDNA was synthesized from total RNA in the presence of random primer p(dN)₆ (Boehringer) using Molo-

neur murine leukemia virus reverse transcriptase SuperScript™ (GIBCO BRL). For all samples, synthesis of cDNA was con-

trolled by RT-PCR using β-actin primers for 30 cycles. CD8α chain mRNA was analyzed using these primers: CAC GAA TAA TAA GTA GTT TCT CAC C (sense) and ATG ATA TCA CAG GCG AAG TCC A (antisense). PCR were per-

formed using 1 IU of Goldstar DNA Taq polymerase (Advanced Biotechnologies), 50 pmol of appropriate primers, 250 µM of each dNTP except for dCTP (3,000 Ci/mmol; NEN Life Sci-

products) in 96-well polycarbonate Costar Thermowell™ strips (Corning) in a PTC-100™ programmable thermal controller (MJ Research, Inc.). 40 cycles of amplifications were performed as follows: 1 min at 94°C, 45 s at 48°C, 1 min at 72°C, followed by 10 min of elongation at 72°C. Samples were sepa-

rated in 2% agarose gels and stained with ethidium bromide.

Results

In Vivo Induction of OVA257–264-specific Cytotoxic Response by PPV-VLPs-OVA. In a previous report (19), we established that PPV-VLPs carrying a CD8⁺ T cell epitope of LCMV (PPV-VLPs-LCMV) induced a strong LCMV-spe-

CIF response. In the present study, PPV-VLPs carrying an H-2b-restricted CD8⁺ T cell epitope from OVA257–264 (PPV-VLPs-OVA) were used. We first tested the ability of PPV-VLPs-OVA to in-

duce a CTL response against OVA257–264 peptide–coated cells. C57BL/6 mice were immunized intraperitoneally with a single injection of 10 µg PPV-VLPs-OVA or control PPV-VLPs in PBS. As shown in Fig. 1 A, 7 d after immunization the mice immunized with PPV-VLPs-OVA developed a strong and specific CTL response against the OVA257–264 epitope, whereas, as expected, mice injected with control PPV-VLPs did not show a significant CTL re-

sponse. Similar CTL responses were obtained after two intraperitoneal injections (with a 21-d interval) or one intravenous injection of PPV-VLPs-OVA (unpublished data). This result confirmed our earlier demonstration of the high im-

munogenicity of PPV particles in the absence of adjuvant. As previously demonstrated (19), the CTL response induced by PPV-VLPs was MHC class I restricted and mediated by

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CD11b were cocultured overnight with 10^5 B3Z cells/well. (E) TAP1

expressed as mean monitored by IL-2 production, measured by a CTLL proliferation assay, and

after collagenase/DNase I digestion cells were stained with MACS ter), or B220
tive FACS® diagrams of purified CD11c

trol PPV-VLPs (left), CD11b

cells, only DCs purified from PPV-VLPs-OVA–injected mice were able to present the OVA_{257-264} epitope, whereas DC11b^+ CD11c^− was >90% (Fig. 1 C). When incubated with B3Z cells, although after overnight in vitro incubation with OVA_{257-264} peptide, they were fully able to stimulate B3Z cells (unpublished data). Thus, in vivo, DCs are the only APC capable of efficiently processing PPV-VLPs-OVA. DCs from mice injected with 10 μg (2.6 pmol/mouse) PPV-VLPs-OVA can also present the OVA_{257-264} peptide, although to a lower extent (unpublished data).

Cytosolic antigens have to be processed in the cytosol of APCs and then transported to the Golgi complex to be presented by MHC class I molecules. TAP molecules are required as an essential step in this transport. Therefore, we analyzed the ex vivo PPV-VLPs-OVA presentation by DCs in TAP1^−/− syngeneic mice. 90 min after PPV-VLPs-OVA intravenous injection, spleen DCs from TAP1^−/− mice failed to present the OVA_{257-264} epitope, whereas spleen DCs from TAP1^+/− mice efficiently stimulated B3Z cells (Fig. 1 E). Although TAP1^−/− cells have a diminished expression of class I MHC molecules (29), their capacity to present peptide–K^b complexes was not essentially affected. Indeed, TAP1^+/− and TAP1^−/− DCs incubated in vitro with the OVA_{257-264} peptide equally presented this epitope (unpublished data). Therefore, in vivo, the processing of
PPV-VLPs-OVA required TAP molecules, i.e., a cytosolic processing in DCs.

**Induction of CTL Response by DCs In Vivo Loaded with PPV-VLPs-OVA.** To establish whether DCs can induce CTL response against the epitope delivered by PPV-VLPs, naive syngeneic mice were immunized with CD11c<sup>+</sup>, CD11b<sup>+</sup>CD11c<sup>-</sup>, or B220<sup>+</sup> spleen cells purified from mice intravenously injected with PPV-VLPs-OVA 90 min before purification. 7 d later, the CTL response against OVA<sub>257–264</sub>-coated target cells was analyzed. As shown in Fig. 2, only mice that received CD11c<sup>+</sup> cells, purified from PPV-VLPs-OVA–injected mice, developed a CTL response against the OVA<sub>257–264</sub> epitope. In contrast, mice that received CD11b<sup>+</sup>CD11c<sup>-</sup> or B220<sup>+</sup> cells purified from the same mice did not develop such a specific CTL response. Therefore, DCs are the only APC involved in the in vivo induction of CTL response by PPV-VLPs-OVA.

**Induction of CTL Response by PPV-VLPs-OVA Does Not Require Cross-Priming.** It is currently assumed that exogenous antigens induce CTL response through the cross-priming of DCs (1, 2) in a process that involves the transfer of cell–associated antigens to DCs. Thus, we next determined whether cross-priming is involved in CTL activation by PPV-VLPs through the capture of PPV-VLPs associated to cells by DCs. β2M<sup>−/−</sup> and β2M<sup>+/+</sup> C57BL/6 mice were intravenously injected with PPV-VLPs-OVA and 90 min later their spleen DCs were purified. In vivo–loaded DCs from both groups of mice were then injected into syngeneic naive β2M<sup>+/−</sup> mice and 1 wk later the CTL response against OVA<sub>257–264</sub> was analyzed (Fig. 3). We reasoned that because β2M<sup>−/−</sup> APCs are unable to present MHC class I–restricted epitopes, a CTL response against the OVA<sub>257–264</sub> epitope could be induced only by transfer of cell–associated antigens from β2M<sup>−/−</sup> to β2M<sup>+/+</sup> DCs. Fig. 3 shows that CTLs against OVA<sub>257–264</sub>-coated target cells were only observed after the injection of DCs from β2M<sup>+/+</sup> mice injected with PPV-VLPs-OVA, but not after the administration of DCs purified from β2M<sup>−/−</sup> mice. These results clearly show that DCs capture exogenous PPV-VLPs-OVA and directly induce CTL response, and exclude that the observed CTL response is due to cross-priming.

**Processing of PPV-VLPs-OVA by Subpopulations of DCs.** In mice, spleen DCs do not constitute a homogeneous cell population. On the basis of the CD8α chain expression, it is now accepted that two major subsets of DCs can be distinguished: CD8α<sup>−</sup> and CD8α<sup>+</sup> DCs. To evaluate the capacity of these DC populations to process and present PPV-VLPs, CD8α<sup>−</sup> CD11c<sup>+</sup> and CD8α<sup>+</sup> CD11c<sup>+</sup> cells were purified. A two-step method was used: first, an enrichment step of DCs by MACS anti-CD11c mAb and then a fluorescent-activated cell sorting, using PE–anti-CD11c and FITC–anti-CD8α. This proceeding allows the recovery of a high number of purified DCs in a short time with few steps and minimal manipulation. As shown in a representative experiment (Fig. 4 A), the purity of both cell populations was always >96%. Both DC subpopulations were equally able to stimulate B3Z cells after in vitro incubation with OVA<sub>257–264</sub> peptide (Fig. 4 B, left), which shows that CD8α<sup>−</sup> and CD8α<sup>+</sup> DCs have the same presentation capacity. These two DC subpopulations were incubated with PPV-VLPs-OVA and as shown in Fig. 4 B (right), CD8α<sup>−</sup> CD11c<sup>+</sup> cells effectively presented the OVA<sub>257–264</sub> epitope whereas CD8α<sup>+</sup> CD11c<sup>+</sup> cells weakly stimulated B3Z cells.

To see if a similar difference could also be observed in vivo, we performed an ex vivo PPV-VLPs-OVA presentation assay. Mice were intravenously injected with 50 μg PPV-VLPs-OVA 90 min and 15 h before DC purification. Splenic CD8α<sup>−</sup> and CD8α<sup>+</sup> DCs from both groups of mice were simultaneously purified and incubated with B3Z. When DCs were purified 90 min after injection, the B3Z hybridoma was selectively stimulated by CD8α<sup>−</sup>-DCs, whereas CD8α<sup>+</sup> DCs had a weak stimulatory activity. In contrast, 15 h after injection, although CD8α<sup>−</sup> DCs were still able to stimulate B3Z cells, a very high stimulation was observed with CD8α<sup>+</sup> DCs (Fig. 4 C). Similar results were obtained in 11 independent experiments confirming that CD8α<sup>−</sup> DCs are capable of presenting PPV-VLPs at early times, but 15 h later CD8α<sup>+</sup> DCs are the
main DC subset capable of presenting this exogenous antigen. Similar results were obtained with DCs from mice injected with 10 μg PPV-VLPs-OVA (unpublished data), although the level of B3Z stimulation was lower. Interestingly enough, under these sub-optimal conditions the presentation by CD8α− DCs at 90 min was rather low compared with the presentation of CD8α+ DCs at 15 h. Both DC subpopulations, purified 90 min or 15 h after PPV-VLPs-OVA injection and cultured overnight with the OVA257–264 peptide (10−1 nM), efficiently stimulated B3Z cells, which shows that these DCs retained the capacity to present antigens at both assayed times (Fig. 4 C, insets). Therefore, these two subpopulations seem to differ in their capacity to take up/process PPV-VLPs, but not in their capacity to present the OVA257–264 epitope delivered by such exogenous antigens.

PPV-VLPs Are Taken by DCs In Vivo. To characterize the in vivo uptake of PPV-VLPs, we labeled PPV-VLPs-OVA with Alexa 488, a strong green fluorescent dye that fluorescence does not extinguish at low pH. After labeling, Alexa 488–PPV-VLPs-OVA maintained their capacity to be processed and presented by DCs to B3Z cells (unpublished data), showing the preservation of their biological activity. We injected Alexa 488–PPV-VLPs-OVA into naïve C57BL/6 mice. 90 min later, their spleen cells were sorted out into CD11c+*, CD11b+, and B220+ subpopulations by magnetic sorting and were labeled with several mAbs. A representative experiment is depicted in Fig. 5 A and summarized in Fig. 5 B. 90 min after injection, only a small fraction of B220+ spleen cells captured Alexa 488–PPV-VLPs-OVA (Fig. 5 A, a). In contrast, half of the CD11c+ cells were Alexa 488+ (Fig. 5 A, b). When CD11b+ cells were isolated and labeled with an anti-CD11c antibody, these two main cell populations were evidenced: one that was strongly Alexa 488+ CD11c+ and another that was Alexa 488low CD11c− (Fig. 5 A, c). These results demonstrated that in the pool of CD11b+ cells, PPV-VLPs are mainly captured by CD11c+ cells (i.e., DCs). Granulocytes, labeled with anti-GR1 mAb, did not show any uptake of Alexa 488–PPV-VLPs-OVA (unpublished data), which suggests that the weak uptake observed in the CD11c− CD11b+ population could be attributed to spleen macrophages. 15 h after injection, DCs remained strongly positive for Alexa 488, whereas B cells were still negative and macrophages were weakly positive for fluorescent VLPs (unpublished data).
anti-CD11c and analyzed on a FACStar™ cytometer. Dot plot analysis of cells obtained were then stained with PE–anti-B220, PE–anti-CD11b, and PE–anti-CD8α molecules. In conclusion, although CD8α− DCs were unable to present PPV-VLP−OVA 90 min after injection, they had already captured them. These results demonstrate that the differences observed in PPV-VLPs-OVA presentation between CD8α− and CD8α+ DCs could not be attributed to a difference in the uptake of PPV-VLPs-OVA.

CD8α− CD11b+ CD11c+ Cells, In Vivo Pulsed with PPV-VLPs-OVA, Express the CD8α Molecule. Up-regulate CD205 and Down-regulate CD4 in Rag2−/− Mice. Considering the different capacity of DC subpopulations to present epitopes delivered by PPV-VLPs at different times, and the decrease of the proportion of CD4+CD8α− DCs observed 15 h after the uptake of Alexa 488–PPV-VLPs-OVA, we analyzed whether these differences could be related to changes in the composition of spleen DC populations. Therefore, we injected 50 μg PPV-VLPs to Rag2−/− mice and 15 h later we recovered the CD11c+ spleen cells for FACS® analysis. After PPV-VLPs injection, no major changes were observed in the percentages of DC subpopulations in a CD11c versus CD8α dot plot, except for a little shift toward the right of the CD8α− subpopulation (Fig. 7 A). However, when CD8α expression was analyzed against CD11b1 expression in CD11c+ cells, a significant number of CD11b+ cells expressed CD8α after PPV-VLPs injection. Furthermore, these CD8α+ CD11b+ CD11c+ cells represented an important percentage of total CD8α+ DCs (Table I). The same analysis performed with another anti-CD8α antibody showed similar results (Fig. 7 A). This CD8α+ CD11b+ DC population was observed until 24 h after PPV-VLPs injection and then disappeared (unpublished data). One possible explanation for this result could be that CD8α+ T cells interacting with DCs remained attached after sorting, giving a false CD8α staining on DCs. However, anti-CD90 antibody (unpublished data) as well as an anti-CD8β antibody did not label DCs (Fig. 7 A), making this hypothesis unlikely. At the same time a strong decrease in the percentage of CD4+ CD11b+ DCs was observed. This modification was accompanied by a diminution in the intensity of CD4 expression. Furthermore, the expression of CD205 was up-regulated after PPV-VLPs injection in CD11b+ DCs. The same analysis performed 90 min after PPV-VLPs-OVA injection showed no change in the expression of CD8α, CD4, or CD205 on CD11b− or CD11b+ DCs compared with noninjected mice (unpublished data).

Considering the different capacity of CD8α− and CD8α+ DCs to present PPV-VLPs-OVA, we investigated whether a differential uptake of PPV-VLPs-OVA by these two DC populations exists. Mice were injected with Alexa 488–PPV-VLPs-OVA and 90 min or 15 h later their spleen DCs were purified and labeled with anti-CD8α and anti-CD4 mAbs to distinguish the three main spleen DC subpopulations: CD4+ CD8α+, CD4+ CD8α−, and CD4− CD8α− DCs (Fig. 6 A). 90 min after Alexa 488–PPV-VLPs-OVA injection, all DC subpopulations in the spleen captured PPV-VLPs and kept the fluorescence for at least 15 h (Fig. 6 B). However, CD4− CD8α+ DCs took slightly more PPV-VLPs than CD8α− DCs. Furthermore, 15 h later we observed a decrease in the percentage and mean fluorescence intensity FL1 of Alexa 488+ CD4+ CD8α− DCs (Fig. 6 C), which was not observed with other DC subpopulations. This decrease correlated with a diminution in the proportion of this subpopulation in the whole spleen DC pool.

In conclusion, although CD8α− DCs were unable to present PPV-VLPs-OVA 90 min after injection, they had already captured them. These results demonstrate that the differences observed in PPV-VLPs-OVA presentation between CD8α− and CD8α+ DCs could not be attributed to a difference in the uptake of PPV-VLPs-OVA.

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<th>Alexa 488–PPV-VLPs</th>
<th>%a</th>
<th>MFIb</th>
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<tbody>
<tr>
<td>CD11c+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD11c CD11b+</td>
<td>63</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>CD11c CD11b+</td>
<td>30</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>B220−</td>
<td>5</td>
<td>25</td>
<td></td>
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</table>

*Percentage of Alexa 488+ cells; b Mean fluorescence intensity of Alexa 488+ cells.

Figure 5. PPV-VLPs-OVA are uptaken in vivo by DCs. (A) One C57BL/6 mouse was intravenously injected with 50 μg Alexa 488–PPV-VLPs. One PBS-injected mouse was used as a control. 90 min later, their spleen cells were obtained and sorted out into B220− (a), CD11c+ (b), or CD11b+ (c) subpopulations by magnetic sorting. All cell populations obtained were then stained with PE–anti-B220, PE–anti-CD11b, and PE–anti-CD11c and analyzed on a FACS® cytometer. Dot plot analysis of B220− (a), CD11c+ (b), and CD11b+ (c) cells purified from PPV-VLPs–injected or control mice and labeled with the indicated PE-coupled mAbs are shown. B220− cells were negative for CD11c and CD11b. The percentage and mean fluorescence intensity of Alexa 488+ cells in CD11c+, CD11b+, and B220− cells in the spleen of Alexa 488–PPV-VLPs-OVA–injected mice, as detailed in A. One representative experiment out of two is depicted.
(unpublished data). To examine whether T cells play a role in these events, we repeated these experiments using Rag2 \(^{-/-}\) mice. In Rag2 \(^{-/-}\) mice, CD11b\(^{+}\) DCs showed no expression of CD8\(\alpha\), but there was a decrease in the percentage of CD8\(\alpha^{+}\) CD11b\(^{-}\) DCs after PPV-VLPs injection (Fig. 7 A and Table I). The percentage of CD4\(^{+}\) DCs was only slightly decreased whereas the expression of CD205 (Fig. 7 A) and MHC class I and II, CD40, and CD80 molecules (unpublished data) showed that they are indeed targeted by PPV-VLPs. The capture of Alexa 488–PPV-VLPs-OVA by CD4\(^{+}\) CD8\(\alpha^{+}\), CD4\(^{+}\) CD8\(\alpha^{-}\), and CD4\(^{-}\) CD8\(\alpha^{+}\) CD11c\(^{+}\) cells purified 90 min and 15 h after injection, was comparable to that observed with normal mice (unpublished data).

The expression of the CD8\(\alpha\) molecule could be attributed to these two different origins: the translation of CD8\(\alpha\) mRNA by CD11b\(^{+}\) DCs or the uptake of CD8\(\alpha\) molecules from other cells, such as CD8\(^{+}\) T cells or CD8\(\alpha^{+}\) CD11b\(^{+}\) DCs. To address this question, we analyzed the expression of CD8\(\alpha\) mRNA by two rounds of RT-PCR

![Image](image_url)

**Figure 6.** In vivo uptake of PPV-VLPs by DC subpopulations. C57BL/6 mice were intravenously injected with 50 \(\mu\)g Alexa 488–PPV-VLPs–OVA, 90 min or 15 h later their CD11c\(^{+}\) spleen cells were purified and stained with PE and APC-coupled mAbs for analysis on a FACScalibur\(\text{®}\) cytometer. Noninjected mice were used as control. (A) CD11c\(^{+}\) spleen cells from control mice were stained with APC–anti-CD8\(\alpha\) and either PE–anti-CD11c (left) or PE–anti-CD4 (right). In this representative experiment, CD11c\(^{+}\) purified spleen cells contained 53\% CD4\(^{+}\) CD8\(\alpha^{+}\), 17\% CD4\(^{-}\) CD8\(\alpha^{+}\), and 20\% CD4\(^{-}\) CD8\(\alpha^{-}\). Similar values were obtained from injected mice. (B) FL1 (Alexa 488) histograms, gated on regions marked on the right panel in A, corresponding to CD4\(^{-}\) CD8\(\alpha^{+}\), CD4\(^{+}\) CD8\(\alpha^{+}\), and CD4\(^{+}\) CD8\(\alpha^{-}\) CD11c\(^{+}\) cells purified 90 min (thick line histograms) or 15 h (gray histograms) after injection or from control (thin line histograms). (C) Percentage and mean fluorescence intensity of Alexa 488\(^{+}\) cells, corresponding to the histograms depicted in B. One representative experiment out of two is depicted.

![Image](image_url)

**Figure 7.** CD11b\(^{+}\) DCs from Rag2\(^{-/-}\) but not from Rag2\(^{+/+}\) mice express CD8\(\alpha\), up-regulate CD205, and down-regulate the CD4 molecules after PPV-VLPs injection. (A) C57BL/6 Rag2\(^{-/-}\) or Rag2\(^{+/+}\) mice were intravenously injected with 50 \(\mu\)g PPV-VLPs. Noninjected mice from each strain were used as a control. 15 h later, CD11c\(^{+}\) spleen cells were purified and stained with anti-CD11b and anti-CD8\(\alpha\) antibodies plus one of the following antibodies: anti-CD11c, anti-CD4, anti-CD205, anti-CD8\(\beta\), or anti-CD86. Cells were analyzed on a FACScalibur\(\text{®}\) cytometer. One representative experiment out of two (for Rag2\(^{+/+}\)) or three (for Rag2\(^{-/-}\)) is depicted. (B) Expression of CD8\(\alpha\) mRNA by DC subgroups. Mice were injected with PPV-VLPs and 90 min later, CD8\(\alpha^{+}\) CD11c\(^{+}\) spleen cells were purified and cultured for 15 h (Exp. 1) or for different times (0, 3, 5, 8, and 15 h, Exp. 2). Then, the mRNA was extracted for RT-PCR (two rounds of 40 cycles per round) using primers for CD8\(\alpha\). mRNA from CD8\(\alpha^{+}\) DCs purified 90 min after injection was used as a positive control.

1240 Presentation of Exogenous Particles by CD8\(\alpha^{-}\) Dendritic Cells to CD8\(^{+}\) T Cells
Table I. CD11b+ DCs Express CD8α in Rag2+/+ Mice Injected with PPV-VLPs-OVA

<table>
<thead>
<tr>
<th>Injection of PPV-VLPs</th>
<th>CD11b+ CD8α+ cells percentage in total CD8α+ DCs</th>
<th>CD11b+ CD8α+ cells percentage in total CD11b+ DCs</th>
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<tbody>
<tr>
<td>Rag2+/+ mice</td>
<td>Rag2+/+ mice</td>
<td>Rag2+/+ mice</td>
</tr>
<tr>
<td>Exp. 1</td>
<td>19 43 5 12</td>
<td>7 15 4 4</td>
</tr>
<tr>
<td>Exp. 2</td>
<td>30 45 17 28</td>
<td>11 23 9 11</td>
</tr>
<tr>
<td>Exp. 3</td>
<td>ND ND 19 22</td>
<td>ND ND 19 9</td>
</tr>
</tbody>
</table>

C57BL/6 Rag2+/+ or Rag2−/− mice were intravenously injected with 50 μg PPV-VLPs. Noninjected mice from each strain were used as a control. 15 h later, CD11c+ spleen cells were labeled with CD11b and anti-CD8α antibodies and analyzed on a FACS calibur® cytometer. At least 2 × 10^4 events were analyzed.

(40 cycles/round) of spleen DC subsets purified 90 min after PPV-VLPs injection and cultured for different times (0, 3, 5, 8, and 15 h). CD8α mRNA was found only in CD8α+ CD11b+ DCs but not in CD11b+ DCs (Fig. 7 B), which shows that CD8α molecules present at the surface of CD11b+ DCs after PPV-VLPs injection did not come from de novo synthesis of this molecule.

**Comparable Presentation of the OVA257–264 Epitope by CD11bhigh DCs 90 min and 15 h after Injection of PPV-VLPs-OVA.** Because some CD11b+ DCs express the CD8α molecule 15 h after PPV-VLPs injection, we analyzed the presentation of the OVA257–264 epitope by these CD8α+ CD11b+ DCs. Because DCs express variable levels of CD11b, it is difficult to define clear-cut subpopulations based on this marker. Therefore, only CD11bhigh DCs were purified from mice injected with PPV-VLPs-OVA 90 min or 15 h earlier (CD11bhigh DCs always contained both CD8α+ and CD8α− cells). CD11bhigh DCs did not express CD8α 90 min after PPV-VLPs-OVA injection, but they expressed this molecule 15 h later (Fig. 8 A). Moreover, CD11bhigh were able to present the OVA257–264 epitope 90 min as well as 15 h after PPV-VLPs-OVA injection (Fig. 8 B). These results show that the CD11b+ DCs that acquired the CD8α molecule were able to present the OVA257–264 epitope.

**Presentation of the OVA257–264 Epitope by CD8α− and CD8α+ DCs from Rag−/− Mice.** Because we did not detect the phenotypic changes in Rag−/− mice injected with PPV-VLPs that we observed in Rag+/+ mice, we analyzed the OVA257–264 epitope presentation by CD8α− and CD8α+ DCs from Rag−/− mice 90 min and 15 h after PPV-VLPs-OVA injection. 90 min after PPV-VLPs-OVA injection, CD8α+ DCs from Rag−/− and Rag+/+ mice were unable to present the OVA257–264 epitope, whereas CD8α− DCs from both strains stimulated the B3Z hybridoma. When the antigen presentation was performed 15 h after PPV-VLPs-OVA injection, the CD8α− DCs from both strains presented the OVA257–264 epitope with an efficacy comparable to the stimulation obtained 90 min after injection (Fig. 8 C). In contrast, CD8α+ DCs from Rag+/+ mice strongly stimulated B3Z cells whereas CD8α− DCs from Rag−/− mice did not exhibit such a dramatic increase in their capacity to stimulate B3Z cells and presented the OVA257–264 epitope with an efficacy similar to CD8α− DCs. These results could suggest that T cells are required for CD8α+ DCs to present the OVA257–264 epitope. Thus, to verify this hypothesis we transferred CD90+ cells (98% T cells, 43% CD4+, 33% CD8+, B220−, CD11b−, CD45RB+, and CD69−; data not shown) purified from Rag+/+ mice to naive Rag−/− mice. 2 d later, we intravenously injected PPV-VLPs-OVA to these mice and tested the ability of CD8α− and CD8α+ DCs to present the OVA257–264 epitope 15 h after injection. Surprisingly, under these conditions, neither CD8α− nor CD8α+ DCs were able to stimulate B3Z cells (unpublished data).

**Discussion**

VLPs clearly demonstrated their potential as vector for vaccination (3, 16–19, 21, 30) and have proven to be a po-

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**Figure 8.** CD11b+ DCs present the OVA257–264 epitope 90 min as well as 15 h after the injection of PPV-VLPs-OVA. Mice were intravenously injected with 50 μg PPV-VLPs-OVA and 90 min or 15 h later, CD11bhigh CD11c+ spleen cells were purified by AutoMACS and a MOFLO® cell sorter. (A) Dot plot analysis of CD11bhigh DCs stained with an anti-CD8α antibody after sorting. (B) Splenic CD11c− (□) and CD11bhigh CD11c+ cells purified 90 min (□) or 15 h (●) after PPV-VLPs-OVA injection were cultured overnight with 10^6 B3Z cells/well. (C) Ex vivo PPV-VLPs-OVA presentation by DC subsets in Rag−/− mice. Naive Rag−/− (●, ○) or Rag−/− (□, □) mice were intravenously injected with 50 μg PPV-VLPs-OVA, 90 min (left) or 15 h (right) before DC purification. CD8α− (●, □) and CD8α+ (○, □) CD11c− as well as CD11c+ (A) cells were simultaneously purified from these mice and cultured overnight with 10^6 B3Z cells/well. The presentation of the SIINFEKL peptide to B3Z cells was monitored by IL-2 production, measured by a CTL proliferation assay, and expressed as mean ± SEM counts per minute (cpm) of duplicate wells. One representative experiment out of two is presented (three pooled mice per group).
tent CTL inducer when compared with other vectors (31). We have developed a VLPs system from PPV that has shown a powerful capacity to elicit both CD4+ (32) and CD8+ (19, 25, 33) T cell responses in the absence of any adjuvant. In this study, we analyzed the in vivo processing and presentation of PPV-VLPs carrying a CD8+ T cell epitope. In vitro, PPV-VLPs-OVA were as efficient as the adjuvant. In this study, we analyzed the in vivo processing and presentation of PPV-VLPs–OVA, only DCs were capable of presenting the OVA257–264 epitope to CD8+ T cells. Additional experiments revealed that PPV-VLPs were efficiently captured by DCs. Finally, only DCs purified from PPV-VLPs–OVA–injected mice could elicit a CTL response. These data clearly demonstrate that in vivo, processing and presentation of PPV-VLPs are performed by DCs.

Several mechanisms responsible for the processing of exogenous antigens in the MHC class I pathway have been described (34). Some are TAP- and proteasome-independent pathways, based on the regurgitation of antigen (35) or the recycling of MHC class I molecules (36) in which antigens are most likely degraded in endosomes and bind to MHC class I molecules without transfer to the cytosol. An alternative pathway, TAP and proteasome dependent, involves the transfer of antigens from phagosomes/macropinosomes to the cytosol, processing by the proteasome complex, and translocation into endoplasmic reticulum/Golgi network using TAP molecules, following the classic MHC class I pathway (37). This last pathway has been shown to be much more efficient than the TAP-independent pathways (38). Some antigens could generate CTL response using simultaneously multiple MHC class I processing pathways (39). In the present study, we demonstrated that although PPV-VLPs are exogenous antigens, they enter into the MHC class I pathway, gain access to cytosol, and are processed by a classic pathway as evidenced by the absence of stimulation of hybridoma cells by PPV-VLPs–OVA–pulsed DCs from TAP1−/− mice and by the requirement for proteasome processing (unpublished data).

Bohm et al. (16) have reported that hepatitis B surface antigen particles are processed by macrophages as well as DCs and that both cell types can prime a CTL response in vivo. However, the processing pathway of these VLPs are clearly different from that of PPV-VLPs. Hepatitis B surface antigen particles bind to recycling rather than new synthesized MHC class I molecules (40). In contrast, PPV-VLPs–derived peptides bind to new, nascent MHC class I molecules, as evidenced by the absence of PPV-VLPs–OVA presentation in TAP−/− mice. DCs, but not macrophages, have the ability to transport the antigens from endosomes to cytosol and then use the cytosolic machinery of processing (41). This may explain why PPV-VLPs, which need cytosolic processing, can only be presented by DCs in vivo.

Cross-priming, a process first described by Bevan (1, 2; for review see reference 42), allows exogenous antigens to elicit a CTL response. The TAP transporter is required for in vivo cross-priming of MHC class I–restricted antigens (43). Hence, considering that PPV-VLPs processing is TAP dependent, we analyzed whether the CTL response induced by PPV-VLPs–OVA is mediated by cross-priming or by direct priming, i.e., by the DC that capture PPV-VLPs–OVA. The transfer of β2M−/− DCs from PPV-VLPs–OVA–injected mice to naïve β2M+/+ mice did not induce a CTL response, whereas the PPV-VLPs–OVA–pulsed β2M+/+ DCs induced such a response. This result clearly shows that DCs initially targeted by PPV-VLPs–OVA are the APCs that induce the CTL response. It should also be noted that the CTL response induced by PPV-VLPs is CD4 independent (19), whereas so far, the CTL responses induced by cross-priming are described to be CD4 dependent (42, 44). Therefore, our data demonstrate that DCs capture exogenous PPV-VLPs–OVA and are able to directly induce a CTL response without cross-priming.

The role of dendritic subsets in the induction of T cell responses is still a matter of intense debate. It has been shown that in mice, splenic CD8α− DCs have the ability to produce large amounts of IL-12 and preferentially induce Th1 responses. By contrast, CD8α+ DCs do not produce large amounts of IL-12 and preferentially induce Th2 responses (45, 46). However, polarization depends upon the site of injection, because the intravenous injection of CD8α+ or CD8α− DCs pulsed with peptide induces non-polarized Th response (47). Concerning the induction of CTL response, one recent study (15) reported that only CD8α+ but not CD8α− DCs from mice injected with OVA-loaded splenocytes can cross-prime CD8+ T cells, although both DC subsets were shown to capture the same amounts of antigen. They suggested that this differential ability could be associated with a different in vivo processing due to a selective capacity of CD8α+ DCs to transport antigen from the endosome to cytosol. Pooley et al. (48) also found that CD8α+ DCs were the principal APC 18 h after an intravenous injection of a high amount of OVA protein. The injection of mice with peptide-pulsed CD8α+ and CD8α− DCs has clearly demonstrated that both DC subsets are equally able to induce a CTL response (47, 49).

In this study we have shown that all DC subsets can capture Alexa 488–PPV-VLPs–OVA to a similar extent, although CD8α+ DCs showed a little higher mean fluorescence intensity than CD8α− DC populations, perhaps because they are slightly larger than CD8α− DCs (49). However, we also showed that shortly after PPV-VLPs–OVA injection, only CD8α− DCs are responsible for the OVA257–264 epitope presentation whereas 15 h later, the presentation is mainly performed by CD8α+ DCs. Thus, these results demonstrated that CD8α− DCs have the capacity to present the OVA257–264 epitope very early after PPV-VLPs capture, whereas CD8α+ DCs presented the OVA257–264 epitope only after a lag time. Because PPV-VLPs are captured efficiently by both subsets, the difference in the accessibility of PPV-VLPs to DCs cannot explain these results. Interestingly, CD8α− from Rag2−/− and Rag2+/+ mice exhibited, at 90 min as well as at 15 h, comparable efficacy to capture and present PPV-VLPs–OVA, which
shows that the processing by this DC subset is T cell independent. In contrast, 15 h after PPV-VLPs-ova injection, CD8α+ DCs from Rag2−/− mice were unable to present the OVA257-264 epitope with the high efficacy of CD8α+ DCs from Rag2+/+ mice. This could suggest that T cells play a role in the licensing of CD8α+ DCs for exogenous antigen presentation. Therefore, our results could suggest that CD8α− and CD8α+ DCs have differential requirements for MHC class I-restricted presentation of exogenous antigens.

Using two different anti-CD8α antibodies, we demonstrated that 15 h after the injection of PPV-VLPs, a significant percentage of CD11b+ DCs expressed CD8α. These cells also expressed CD205. Moreover, the proportion of CD4+ CD8α− DCs showed an important decrease at that time, associated with a diminution in the intensity of expression of the CD4 molecule on those cells. Our study is the first in vivo report showing the apparition of a CD8α+ and CD205+ CD11b+ DC population in the spleen after the injection of an antigen. A very recent report suggests that CD8α+ DCs could originate from the CD8α− DC subset by a maturation process involving CD8α, DEC-205, and CD24 up-regulation 18 h after CD8α− DC transfer (50). However, in that study, the phenotypic change was observed after cell transfer without any stimulation, whereas in our case it was induced by the injection of an antigen. An association of some T cells with DCs, which could eventually explain our results, is excluded because: (a) all purification steps included EDTA, which disrupts interactions between cells, (b) no CD8α mRNA was detected in sorted CD8α− DCs, and (c) anti-CD3e, anti-CD8β, and anti-CD90 antibodies did not specifically bind to purified CD11b+ DCs. A population of CD11b+CD11c+ that expressed CD8α in mice treated with Flt3L was described in two previous reports (51, 52). However, in this study the CD8α+ population is CD11b+, indicating that these populations are different.

The absence of CD8α mRNA in CD11b+ DCs harvested at various times after PPV-VLPs injection suggests that CD8α expression on these cells is not due to de novo synthesis. The lack of expression of the CD8α molecule on CD11b+ DCs from Rag2−/− mice injected with PPV-VLPs, as well as the slight reduction in CD4 expression and the small increase of CD205, also suggests that T cells may play a role in these phenotypic changes. Indeed, the similar uptake of Alexa 488–PPV-VLPs–ova in Rag2+/+ and Rag2−/− mice (unpublished data), as well as the up-regulation of CD86 in DCs of Rag2−/− mice, excluded the possibility that these effects were due to a lack of accessibility of VLPs to DCs from Rag2−/− mice. It remains to be determined if the dramatic increase of CD8α+ presentation observed 15 h after PPV-VLPs–ova injection was due to the CD11b+ CD8α+ or to the CD11b− CD8α+ population, which was inefficient at 90 min and therefore required longer times than CD8α− DCs for VLPs processing.

This study clearly demonstrates that CD8α− DCs have the capacity to transfer the VLPs to the cytotoxic pathway and present these exogenous antigens without cross-priming almost immediately after antigen uptake and independently of T cells. In contrast, CD8α+ DCs cannot present PPV-VLPs immediately after capture, but exhibited a very strong capacity to present the OVA257-264 epitope carried by VLPs at longer times after VLPs capture. The fact that in Rag2−/− mice the lack of expression of CD8α by CD11b+ DCs was accompanied by the inability of CD8α+ DCs to acquire the same antigen–presenting capacity than in Rag2+/+ mice suggests that both events are closely linked.

In conclusion, this study highlighted the specialization of the various DC subsets. Our results, which show that CD8α− DCs can acquire molecules such as CD8α and CD205 after activation, strongly support the view that in vivo studies addressing the functions of DC subsets must define DC subsets carefully, based on the analysis of various markers.

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