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Dendritic Cells Cross-Dressed with Peptide MHC Class I Complexes Prime CD8+ T Cells

Brian P. Dolan, Kenneth D. Gibbs, Jr., and Suzanne Ostrand-Rosenberg

The activation of naive CD8+ T cells has been attributed to two mechanisms: cross-priming and direct priming. Cross-priming and direct priming differ in the source of Ag and in the cell that presents the Ag to the responding CD8+ T cells. In cross-priming, exogenous Ag is acquired by professional APCs, such as dendritic cells (DC), which process the Ag into peptides that are subsequently presented. In direct priming, the APCs, which may or may not be DC, synthesize and process the Ag and present it to themselves to CD8+ T cells. In this study, we demonstrate that naive CD8+ T cells are activated by a third mechanism, called cross-dressing. In cross-dressing, DC directly acquire MHC class I-peptide complexes from dead, but not live, donor cells by a cell contact-mediated mechanism, and present the intact complexes to naive CD8+ T cells. Such DC are cross-dressed because they are wearing peptide-MHC complexes generated by other cells. CD8+ T cells activated by cross-dressing are restricted to the MHC class I genotype of the donor cells and are specific for peptides generated by the donor cells. In vivo studies demonstrate that optimal priming of CD8+ T cells requires both cross-priming and cross-dressing. Thus, cross-dressing may be an important mechanism by which DC prime naive CD8+ T cells and may explain how CD8+ T cells are primed to Ags that are inefficiently cross-presented. The Journal of Immunology, 2006, 177: 6018–6024.

Antigen presentation to CD8+ T lymphocytes is postulated to occur through two mechanisms, which differ from each other by the type of cell that processes and presents the peptide Ag. According to the direct Ag presentation mechanism, CD8+ T cells recognize their cognate peptide-MHC class I complexes on the surface of the cells that synthesized the Ag, such as virally infected cells or malignant cells (1). Alternatively, CD8+ T cells recognize peptide-MHC class I complexes on the surface of cells, which have captured exogenously synthesized Ag, and subsequently process and present the Ag bound to their own MHC class I proteins. This latter mechanism is termed cross-presentation, and dendritic cells (DC) are the predominant cell population that captures and presents Ag (2, 3). If the process of cross-presentation results in the activation of naive T cells, then the T cells have been activated by cross-priming.

Although compelling evidence supports cross-priming as the principal mechanism for activating naive CD8+ T cells (2, 4, 5), other studies are consistent with CD8+ T cells being activated by the direct presentation mechanism (6, 7). The concept of cross-priming is supported by many reports demonstrating that CD8+ T cells specific for virally infected cells are primed by DC cross-presenting virally derived peptides (8–11), although virally infected DC also directly present viral Ags to CD8+ T cells (12, 13). Ultimately, CD8+ T cell priming to viral infections may be the result of both cross-priming and direct priming (14). Cross-priming has also been demonstrated for CD8+ T cells activated to tumor Ags (15–17); however, other studies suggest that priming of tumor-specific CD8+ T cells occurs through the direct presentation of tumor Ags by tumor cells (18, 19). Therefore, a consensus model for CD8+ T cell priming does not exist.

In this study, we report that CD8+ T cells can be primed by a third mechanism, termed cross-dressing. During cross-dressing, peptide-MHC class I complexes are transferred to DC from dead donor cells that synthesized the complexes. Recipient DC then use the acquired peptide-MHC class I complexes to activate CD8+ T cells that are peptide specific and restricted to the MHC class I genotype of the donor cells. Optimal priming of CD8+ T cells probably results from a combination of cross-dressing and cross-priming, because cross-dressing efficiently activates DC that are not efficiently activated by cross-priming.

Materials and Methods

Mice

Breeding stocks of BALB/c, C57BL/6, FVB, OT-I, and CD11c-diphtheria toxin receptor (DTR) (Itgax-DTR/enhanced GFP) mice on a BALB/c background were from The Jackson Laboratory. MHC I/II-/- mice and OT-I/RAG 2-/- mice were from Taconic Farms. CD11c-DTR mice were screened, as described (20). OT-I mice were identified by immunofluorescent staining of FBLNs for CD8 and Vα2. H-2b/CD11c-DTR-/- mice were generated by crossing CD11c-DTR mice with MHC class I/II-deficient mice, and the CD11c-DTR+/- F1 offspring were backcrossed to MHC class I/II-deficient mice. CD11c-DTR+/- F2 mice that were negative for both MHC class I and II (identified by immunofluorescence staining for the absence of peripheral CD4+ and CD8+ T cells) were then crossed with C57BL/6 mice. The F1 mice of this cross were subsequently used in experiments. All animal procedures were approved by the University of Maryland Baltimore County Institutional Animal Care and Use Committee.

Cells, transfections, and Abs

B16BL6 8.2, A20, EL4, and EL4/OVA were cultured, as described (20–22). NIH3T3 cells were grown in DMEM supplemented with 10% FCS.
(HyClone) DAP/OVA cells (23) were maintained in RPMI 1640 (Biofluids) supplemented with 10% FCS, 1 mM HEPES (In vitro Life Technologies), 5 × 10^{-5} M 2-ME (Sigma-Aldrich), 400 μg/ml G418 (Calbiochem), and 200 μg/ml zeocin (Invitrogen Life Technologies). Medium for all cell lines contained 1% penicillin, 1% streptomycin, 1% gentamicin (Biofluids), and 1% Glutamax (Invitrogen Life Technologies) (complete medium). The DAP/OVA/Kc cell line was generated by transfection using Lipofectamine Plus (Invitrogen Life Technologies), according to the manufacturer’s recommendations. Approximately 4 μg of the Kc plasmid pKbT8 (24, 25) was linearized with PvuI (Fermentas Life Sciences), eth- anal precipitated, resuspended in 100 μl of DMEM (Biofluids), mixed with 16 μl of Plus reagent, and incubated for 15 min at room temperature. One hundred microliters of DMEM containing 10 μl of lipofectamine (Biofluids) was then added, and the mixture was incubated for an additional 15 min. The DNA mixture was then added to DAP/OVA cells plated the previous night at 4 × 10^6 cells/well of a 6-well plate, in a final volume of 0.4 ml of DMEM. Cells were cultured with DNA for 3 h before the addition of 0.2 ml of complete medium and an additional 0.6 ml of FCS. H-2Kb^+ cells were identified by staining with fluorescently tagged mAb (clone AF6-88.5; BD Pharmingen). Bulk H-2Kb^+ transfecants were fluorescently sorted using a Coulter Epics Altra cell sorter. Fibroblasts were generated as follows: minced tail tissue was resuspended in 2.5 ml of a 2 mg/ml collagenase IV (Worthington Biochemical) solution supplemented with 10 μl of elastase (MP Biomedicalis), shaken gently for 1–2 days at 4°C, and washed with PBS, and adherent cells were cultured in complete IMDM supplemented with 10% FCS. The following Abs directly coupled to FITC or PE were purchased from BD Pharmingen: CD11c (HL3), CD8 (53-6.7), CD4 (GK1.5), CD69 (H.12F3), K^b (36-7.5), K^d (APF-88.5), D^b (34-2-12S), and Vav2 (B20.1). Anti-OVA mAb (clone 14) and anti-β-tubulin mAb (Tab 2.1) were from Sigma-Aldrich. Sheep anti-mouse HRP Ab was from Amersham.

Western blotting

Western blots for β-tubulin were performed, as described (26). Immunoblotting for OVA followed the same experimental procedure, except the primary Ab for OVA (mAb clone 14) was diluted 1/5000 in 0.5% Tween 20-TBS solution containing 0.5% nonfat milk.

DC isolation and culture

Splenic DC were isolated and cultured, as described (20), using CD11c microbeads (Miltenyi Biotec). Bone marrow-derived DC (BMDC) were isolated by flushing the bone marrow from femurs of 3- to 6-mo-old mice, followed by RBC depletion using ACK lysing buffer (Biofluids). Cells isolated by flushing the bone marrow from femurs of 3- to 6-mo-old mice, were cultured in DC medium (complete RPMI 1640 supplemented with 5% FCS, 1 mM HEPES, 5 × 10^{-5} M 2-ME, 20 ng/ml GM-CSF, and 10 ng/ml IL-4 (RDI)). Medium was changed every 2 days, and cells were 80–90% medium and an additional 60% were cultured with DNA for 3 h before the addition of 2 ml of complete medium supplemented with 2.5 μM CFSE (Molecular Probes) in PBS at 10^7 cells/ml. The reaction was quenched by the addition of 1 ml of FCS, and the cells were washed with excess PBS. A total of 5 × 10^5 labeled cells was injected i.v. into the tail vein of recipient mice, and 8–24 h later mice were inoculated s.c. in the lateral, lower abdominal region with 5–10 × 10^6 live, freeze-thawed, or a 1:1 mixture of live and freeze-thawed cells. Three days later, mice were sacri- ficied, the draining lymph node (superficial inguinal) was removed and dissociated, and the cells were examined by flow cytometry for dilution of CFSE. Background auto fluorescence was determined using mice that did not receive CFSE-labeled cells and was gated out. The proliferation index was calculated by dividing the total number of CFSE^+ cells in all divisions by the number of parental cells originally present, and was calculated using ModFit software (Verity Software House) (29). In CD11c depletion experi- ments, (C57BL/6 × CD11c-DTR-F) mice were injected i.p. with 3 ng/g body weight diphtheria toxin (DTx; Sigma-Aldrich), and 6 h later, inoculated with live and/or freeze-thawed donor cells.

Statistical analysis

SDs and Student’s t test were calculated using Microsoft Excel 2002.

Results

MHC class I molecules transfer from dead cells to DC

Acquisition of MHC class I by DC was first assessed in vitro using BMDC and allogeneic donor cells. BMDC from FVB (H-2K^d) mice were inoculated with 3 h with freeze-thawed genetically mismatched DAP/OVA (H-2^d) fibroblasts, and the resulting cells were purified by passage over a Ficol gradient. Ficol-purified cells (Fig. 1A, top left panel) bitmap to a different location than freeze-thawed cells (Fig. 1A, bottom left panel), are >93% viable (Fig. 1A, middle panel), and are >90% CD11c^+ (right panel), identifying them as DC. To determine whether MHC class I transfers to DC, purified cells were stained for donor genotype H-2K^d molecules. As seen in Fig. 1B (top left panel), H-2^d DC incubated with freeze-thawed DAP/OVA cells stain for H-2K^d, demonstrating that the DC have acquired MHC class I molecules. Similar results were obtained if MHC class I-deficient BMDC were used as recipient DC instead of FVB BMDC (data not shown). MHC class I molecules also trans- ferred to DC from apoptotic DAP/OVA cells (data not shown). In agreement with previous studies of MHC class II transfer to DC (20), MHC molecules are not detected on BMDC cultured with live DAP/OVA cells (Fig. 1B, bottom left panel).

To test whether MHC class I molecules were retained during DC maturation, DC were cocultured with freeze-thawed DAP/ OVA cells, purified by Ficol gradient, and cultured overnight in the presence of LPS and mAbs to CD40 to induce maturation. The following day, BMDC were stained for donor MHC class I (H- 2K^d). As shown in Fig. 1B (bottom right panel), donor H-2K^d protein is detected after overnight culture and DC maturation, demonstrating the stability of transferred MHC class I molecules.

To ensure specificity of MHC class I transfer, K^b-expressing DAP/OVA cells were generated (DAP/OVA/K^b; Fig. 1C), and we...
for H-2Kk. As shown in Fig. 1, FVB BMDC. Three hours later, the DC were purified and labeled min, and the supernatant was removed and added to cultures of OVA/Kb vs DAP/OVA cells. H-2Kb was detected on FVB BMDC separating DC from freeze-thawed DAP/OVA cells. DC were subsequently cultured with the supernatant of freeze-thawed DAP/OVA cells, or with a Transwell. FVB BMDC were cultured alone, with freeze-thawed DAP/OVA cells, or with H-2Kb-transfected parental DAP/OVA cells (Fig. 1C). These data are representative of three independent experiments.

MHC class I transfer requires cell-to-cell contact

To determine whether the particulate material or the supernatant of the freeze-thawed cells was involved in MHC class I transfer, freeze-thawed DAP/OVA cells were centrifuged at 300g for 3 min, and the supernatant was removed and added to cultures of FVB BMDC. Three hours later, the DC were purified and labeled for H-2Kk. As shown in Fig. 1E, MHC class I transfer in the presence of the supernatant is ~30% of the level obtained with nonfractionated freeze-thawed donor cells. To test whether cell contact between dead cells and DC is required for MHC class I transfer, FVB BMDC were separated from freeze-thawed DAP/OVA cells for the 3-h culture period using an 8.0-μm Transwell, and then stained for H-2Kk. Under these conditions, MHC class I transfer is <25% of the level when freeze-thawed cells and DC are mixed. To ensure that small molecules could traverse the Transwell membrane, 50-nm FITC-coated latex beads were mixed with the freeze-thawed cells and added to one side of the Transwells. After 3 h of culture, BMDC were analyzed for bead uptake. Only a partial reduction of bead uptake was observed (~29%; data not shown), indicating that small materials readily traversed the membrane. Collectively, these data suggest that optimal MHC class I transfer to DC requires direct contact with dead cells or large cellular debris, and is not efficiently mediated by soluble molecules or small vesicles such as exosomes (30–32) bearing MHC class I peptide complexes.

MHC class I transfers from a variety of cells

To determine whether DC acquisition of MHC class I was a general phenomenon, transfer experiments were conducted with the B16.BL6 8.2 melanoma (H-2Kk) and the A20 B lymphoma (H-2d) cell lines. As seen in Fig. 2A, H-2Kk molecules from the melanoma cell line and H-2Dd molecules from the B cell lymphoma were detected on BMDC from MHC-mismatched (FVB) mice. Modest transfer to FVB BMDC was even seen from primary fibroblasts derived from the tail tissue of BALB/c (H-2k) mice (Fig. 2A, right panel). These data in combination with previously published data (20) demonstrate that a wide variety of cell types can donate MHC class I molecules to DC.

To determine whether the levels of MHC class I on the donor cell affected the efficiency of transfer, EL4/OVA cells (H-2k), which express very low levels of H-2Kk, were treated with IFN-γ to up-regulate MHC class I expression (Fig. 2B). Freeze-thawed IFN-γ-treated or untreated EL4/OVA cells were then cultured with FVB-derived BMDC. Transfer of H-2Kk molecules from freeze-thawed IFN-γ-treated EL4/OVA cells was greater than from

FIGURE 1. MHC class I molecules transfer from donor cells to DC. A, FS × SS log profile, and propidium iodide and CD11c staining of bit-mapped FVB (H-2d) BMDC cultured for 3 h with freeze-thawed DAP/OVA cells, purified by passage over a Ficoll gradient (top row). FS × SS log and propidium iodide staining of freeze-thawed DAP/OVA cells (bottom row). B, FVB BMDC were cultured alone or with freeze-thawed DAP/OVA cells, and analyzed for H-2Kk (right histogram, isotype control staining). BMDC were incubated alone or with live DAP/OVA cells, and analyzed for H-2Kk (left histogram). FVB BMDC were cultured alone or with freeze-thawed DAP/OVA cells, purified, cultured overnight with LPS and anti-CD40 mAbs, and analyzed for H-2Kk expression the following day. C, DAP/OVA or H-2Kk-transfected DAP/OVA (DAP/OVA/Kk) cells cultured for 3 h with H-2Kk. D, FVB BMDC were cultured alone or with freeze-thawed DAP/OVA cells, or with DAP/OVA/Kk (right histogram) cells, purified, and analyzed for H-2Kk. E, FVB BMDC were cultured alone, with freeze-thawed DAP/OVA cells, with the supernatant of freeze-thawed DAP/OVA cells, or with a Transwell separating DC from freeze-thawed DAP/OVA cells. DC were subsequently analyzed for H-2Kk. All BMDC were purified by passage over Ficoll gradients before Ab staining. BMDC histograms show the Ab staining for CD11c+ cells. These data are representative of three independent experiments.

FIGURE 2. MHC class I molecules transfer to DC from a variety of cells. A, FVB BMDC were incubated alone or with freeze-thawed B16, A20, or BALB/c fibroblast cells, and analyzed for donor MHC class I. B, EL4/OVA or IFN-γ-treated EL4/OVA cells stained for H-2Kk. C, FVB BMDC were incubated alone or with freeze-thawed EL4/OVA or IFN-γ-treated EL4/OVA cells and analyzed for H-2Kk. These data are representative of three independent experiments.
freeze-thawed untreated cells (Fig. 2C). Similarly, H-2Dd transfer from IFN-γ-treated A20 cells to FVB BMDC was increased compared with non-IFN-γ-treated A20 cells (data not shown). Increased transfer of H-2Kb from IFN-γ-treated EL4/OVA cells to splenic DC was also observed (data not shown). Therefore, increased expression of MHC class I on donor cells leads to enhanced acquisition of MHC class I proteins by DC.

Transferred peptide-MHC class I complexes activate CD8⁺ T cells

The MHC class I molecules acquired by DC may activate T cells. To test this hypothesis, FVB BMDC were cultured with freeze-thawed EL4 cells transfected with the OVA gene (EL4/OVA), purified, and tested as APCs with OVA-specific, H-2Kb-restricted, OT-I transgenic CD8⁺ T cells. BMDC exposed to EL4/OVA, but not EL4 or OVA protein, activated OT-I T cells, as measured by IL-2 release (Fig. 3A). Similar results were obtained using splenic DC (data not shown). Therefore, DC acquire MHC class I molecules and Ag from donor cells, and use the acquired material to prime CD8⁺ T cells.

T cell activation in Fig. 3A could be due to the transfer of MHC class I-OVA complexes formed in the donor EL4/OVA cells, or to the independent transfer of MHC class I and Ag and the subsequent assembly of MHC class I-peptide complexes in the recipient DC. To distinguish these two possibilities, FVB BMDC were cultured for 3 h with either freeze-thawed EL4/OVA cells or a combination of freeze-thawed EL4 cells and OVA protein. BMDC were purified and then tested for their ability to activate OT-I T cells. OT-I T cell activation only occurred if EL4 cells contained OVA (Fig. 3B), indicating that peptide-MHC I complexes are generated in the donor cells before transfer, and that the transferred complexes are presented by DC without further processing.

Cross-dressed DC efficiently activate CD8⁺ T cells

We reported similar transfer of peptide-MHC II complexes in a previous study, and referred to the process as DC cross-dressing (20), a term originally coined by Yewdell and Haeryfar (33). To determine the role of cross-dressing in CD8⁺ T cell priming, T cells were activated under conditions in which the relative contributions of cross-priming and cross-dressing could be assessed. C57BL/6 (H-2b) BMDC were cultured with freeze-thawed DAP/OVA or DAP/OVA/Kb cells, purified by passage over Ficoll, and cultured with OT-I T cells. OT-I T cell activation was measured by assessing expression of the early activation marker CD69 (34) and by production of IL-2 and IFN-γ. BMDC exposed to freeze-thawed DAP/OVA/Kb cells induced a 4-fold increase in the percentage of CD69⁺ OT-I T cells relative to BMDC exposed to freeze-thawed DAP/OVA cells (Fig. 4A), whereas OT-I T cells

FIGURE 3. Peptide-MHC class I complexes transfer from donor cells to DC and activate CD8⁺ T cells. A, FVB BMDC were cultured alone, with freeze-thawed EL4 or EL4/OVA cells, or with 10 μg/ml OVA, purified by passage over a Ficoll gradient, and cultured overnight with OT-I T cells. IL-2 release was quantified by ELISA. B, FVB BMDC were cultured with freeze-thawed EL4/OVA cells or with freeze-thawed EL4 cells and 10 μg/ml OVA, purified, and cultured overnight with OT-I T cells. IL-2 release was quantified by ELISA. These data are representative of three independent experiments.

FIGURE 4. Cross-dressed DC activate naive CD8⁺ T cells. C57BL/6 BMDC cultured with freeze-thawed DAP/OVA cells, DAP/OVA/Kb cells, or without fibroblasts were purified on Ficoll gradients and cultured with OT-I T cells. OT-I T cell activation was measured by CD69 expression at the indicated times (A), or by IL-2 (B) or IFN-γ (C) production the following day. D, DAP/OVA and DAP/OVA/Kb cells have similar levels of cell-associated OVA, as determined by Western blot. These data are representative of three independent experiments.
cultured alone were <1% CD69⁺ (Fig. 4A). IL-2 and IFN-γ production was similarly higher in cultures with DC exposed to freeze-thawed DAP/OVA/Kb cells (Fig. 4, B and C, respectively). No IL-2 release was observed when OT-I T cells were cultured with freeze-thawed DAP/OVA/Kb cells in the absence of DC (Fig. 4B), indicating that DC are essential for CD8⁺ T cell activation and that OT-I activation is not due to direct priming by the freeze-thawed DAP/OVA/Kb cells. Comparable results were obtained if splenic DC were used as recipients, although fewer T cells expressed CD69 and both IL-2 and IFN-γ release was lower (data not shown). To determine whether the differences in T cell activation were due to quantitative differences in cell-associated OVA, cell lysates of DAP/OVA/Kb and DAP/OVA cells were prepared and Western blotted with Abs to OVA. As shown in Fig. 4D, DAP/OVA cells contain more OVA than DAP/OVA/Kb cells, indicating that the enhanced activation of OT-I T cells by DC cocultured with freeze-thawed DAP/OVA/Kb cells is not due to differences in quantity of OVA protein.

The absence of significant T cell activation by DAP/OVA-loaded DC vs the strong T cell activation by DAP/OVA/Kb-loaded DC (Fig. 4) is consistent with the hypothesis that cross-dressing is more efficient than cross-priming for low levels of soluble Ag. To explore this possibility, C57BL/6 BMDC were pulsed for 3 h with soluble OVA at concentrations comparable to OVA levels in EL4/OVA lysates (0.1 μg/ml), or with 100-fold excess OVA (10 μg/ml). The protein-pulsed DC were then compared with EL4 and EL4/OVA-loaded DC for their ability to activate OT-I T cells. EL4/OVA-loaded DC and DC pulsed with 100-fold excess OVA significantly activate OT-I T cells; however, DC pulsed with 0.1 μg/ml OVA do not activate (Fig. 5). Therefore, cross-dressing is more efficient than cross-priming when Ag levels are low.

**DC cross-dressing primes CD8⁺ T cells in vivo**

To determine whether CD8⁺ T cells are primed in vivo by cross-dressed DC, C57BL/6 mice were adoptively transferred with CFSE-labeled OT-I T cells and inoculated s.c. with DAP transfectants. Three days later, the draining lymph node was removed and examined for dilution of CFSE. Each histogram shows the result of an individual mouse. The average proliferation index ± SD for three mice tested in independent experiments is shown in the top left corner of each histogram. Proliferation indices marked with an * are significantly different from the unmarked indices (p < 0.05) and are not significantly different from each other. A, CFSE staining for C57BL/6 mice inoculated with a 1:1 mixture of live and freeze-thawed (dead) DAP/OVA/Kb or DAP/OVA cells (left and middle panels) or CD11c-DTR (H-2b) mice depleted for DC by DTx injection and inoculated with a 1:1 mixture of live and dead DAP/OVA/Kb cells (right panel). B, CFSE staining for C57BL/6 mice inoculated with live or dead DAP/OVA/Kb cells, or a 1:1 mixture of live and dead DAP/OVA/Kb cells. C, CFSE staining for C57BL/6 mice inoculated with a 1:1 mixture of live and dead DAP/OVA, DAP/OVA/Kb, or NIH3T3 cells.
(H-2b), which, when treated with DTx, are transiently depleted for CD11c expressing DC (35), were used. CD11c-DTR mice were DTx treated, adoptively transferred with CFSE-labeled OT-I T cells, and inoculated with a mixture of live and freeze-thawed DAP/OVA/Kb cells, and 3 days later the draining lymph node was examined for expansion of OT-I T cells. Deletion of CD11c+ cells virtually eliminated expansion of OT-I T cells (Fig. 6A, right panel), demonstrating that DC are essential for T cell priming and that DAP/OVA/Kb fibroblasts cannot directly prime OT-I T cells. In contrast, OT-I expansion in nondiphtheria-treated CD11c-DTR transgenic mice inoculated with DAP/OVA/Kb cells, and in nontransgenic mice inoculated with DAP/OVA/Kb and treated with DTx was similar to expansion in Fig. 6A, left panel (data not shown). Therefore, CD11c+ cells are required for in vivo T cell priming, demonstrating that DAP/OVA/Kb cells do not directly present Ag, and supporting the conclusion that cross-dressing occurs in vivo and enhances T cell priming compared with cross-priming alone. Although OT-I T cells expand in mice inoculated with a mixture of live and freeze-thawed (dead) DAP/OVA/Kb cells, inoculation with exclusively live or freeze-thawed DAP/OVA/Kb cells did not induce significant OT-I T cell division (Fig. 6B), suggesting synergy between the live and freeze-thawed cell populations. To determine which cell population was providing the peptide-MHC complexes for cross-dressing, C57BL/6 mice were adoptively transferred with OT-I T cells and inoculated with 1:1 mixtures of live and freeze-thawed (dead) DAP/OVA, DAP/OVA/Kb, and NIH3T3 cells. OT-I T cells expanded in mice inoculated with a mixture of live DAP/OVA and dead DAP/OVA/Kb cells (Fig. 6C, left panel), but not in mice inoculated with mixtures of live DAP/OVA/Kb plus dead DAP/OVA cells, or mixtures of live NIH3T3 plus dead DAP/OVA/Kb cells (Fig. 6C, middle and right panels). Therefore, T cell expansion requires live and dead cells, and the dead cells must contain the relevant peptide-MHC class I complexes, whereas the live cells must contain the relevant Ag. As DC acquire peptide-MHC class I complexes from dead and not live cells (Figs. 1 and 3), the dead cell population is probably inducing DC cross-dressing, whereas the live population is inducing cross-priming. Therefore, maximum priming of CD8+ T cells is probably the result of a combination of DC cross-dressing and cross-priming.

Discussion

The priming of CD8+ T cells by cross-dressed DC is a newly identified mechanism by which CD8+ T cells are activated. Although there has been speculation that cross-dressing occurs (33), this is the first report demonstrating that it takes place in vitro and in vivo. Cross-priming and cross-presentation, the generally accepted mechanisms for activating CD8+ T cells, differ significantly from cross-dressing. Cross-dressed DC acquire peptide-MHC class I complexes from exogenous sources and present the complexes without further processing. Although DC also acquire Ag from exogenous sources during cross-priming/presentation, they process the acquired Ag intracellularly and present it in the context of their MHC class I molecules. Therefore, cross-priming activates CD8+ T cells that are restricted to the MHC genotype of the DC and specific for peptides generated by the DC, whereas cross-dressing activates CD8+ T cells that are restricted to the MHC genotype of the donor cells and are specific for peptides generated by the donor cells. Several reports suggest that cells other than DC, such as tumor cells or fibroblasts, can directly activate CD8+ T cells (6, 18, 19). These studies used mice inoculated with genetically mismatched cells or recipient mice deficient in TAP so as to prevent cross-priming. However, DC were present in recipient mice and therefore could have been cross-dressed with peptide-MHC class I complexes from the inoculated cells. Therefore, to definitively demonstrate direct Ag presentation to CD8+ T cells by nonprofessional APCs, these studies should be done under conditions that prevent DC cross-dressing.

Although cross-priming and cross-presentation have been experimentally documented for many Ags, including soluble proteins and viral and tumor Ags (reviewed in Refs. 2 and 5), not all CD8+ T cell determinants are efficiently cross-presented (13, 18, 36, 37), suggesting that additional activation mechanisms are operating. Although direct presentation has been proposed as the relevant mechanism for epitopes that are inefficiently cross-presented (14), cross-dressing may be responsible. This conclusion is supported by the studies reported in this work, demonstrating that cross-dressed DC prime CD8+ T cells when cross-priming is inefficient. Although the relative contributions of cross-dressing and cross-priming to overall CD8+ T cell activation may vary for different Ags, it is likely that a combination of the two mechanisms facilititates maximum CD8+ T cell activation.

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