The Journal of Immunology

Dendritic Cells Use Endocytic Pathway for Cross-Priming Class Ib MHC-Restricted CD8 $\alpha\alpha^{+}$TCR$\alpha\beta^{+}$ T Cells with Regulatory Properties

Trevor R. F. Smith, Xiaolei Tang, Igor Maricic, Zacarias Garcia, Shaohsuan Fanchiang and Vipin Kumar

J Immunol 2009; 182:6959-6968; ;
doi: 10.4049/jimmunol.0900316
http://www.jimmunol.org/content/182/11/6959

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Dendritic Cells Use Endocytic Pathway for Cross-Priming Class Ib MHC-Restricted CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T Cells with Regulatory Properties[1]

Trevor R. F. Smith,* Xiaolei Tang,[2]* Igor Maricic,* Zacarias Garcia,† Shaohsuan Fanchiang,* and Vipin Kumar[3]*

Understanding the mechanisms leading to effective priming of lymphocytes with regulatory properties is crucial for the manipulation of immune responses. CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells are a special subset of innate-like lymphocytes that have been shown to be involved in immune regulation. These cells can recognize self-peptides in the context of a class Ib molecule, Qa-1. How self-Ags are processed in the Qa-1 pathway and presented to CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells is not understood. In this study we demonstrate a cross-presentation pathway by which bone marrow-derived dendritic cells (DCs) capture apoptotic CD4$^+$ T cells and process and present TCR-derived peptides in the context of Qa-1 to prime CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells. The priming ability of the DCs is enhanced following TLR signaling using TLR3, TLR4, and TLR9 agonists. DC-mediated cross-presentation is inhibited in the presence of endosomal and proteasomal Ag-processing antagonists. Importantly, DCs loaded with apoptotic T cells prime CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells in vivo, which in turn provides protection from CD4$^+$ T cell-mediated autoimmune disease. These data provide a key insight related to processing and presentation of self-Ags in the Qa-1 pathway for priming of CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells and have implications for a DC-based immunotherapeutic approach to inflammatory diseases. ***The Journal of Immunology,*** **2009, 182: 6959–6968.**

Major histocompatibility complex class Ib molecules, including Qa-1, Qa-2, H2-M3, and CD1d in rodents and HLA-E, HLA-G, and CD1 in humans, differ from MHC class Ia molecules in that they have limited polymorphism (1). Class Ib molecules associate with β_2-microglobulin and present Ags to CD8$^+$ T cells. MHC class Ib-restricted CD8$^+$ T cells in rodents and humans can recognize self-Ags as well as Ags derived from a number of pathogens (2, 3). For example, H2-M3- and HLA-E-restricted CD8$^+$ T cells can recognize *Mycobacterium tuberculosis* Ags (4, 5), whereas Qa-1-restricted CD8$^+$ T cells are abundant in *Salmonella typhimurium*- and *Listeria monocytogenes*-infected mice (6, 7). Class Ib-restricted CD8$^+$ T cells are also involved in antitumor immunity (8–10) and in immune regulation mechanisms (11–13). MHC class Ib-restricted T cells are distinct from MHC class Ia-restricted T cells in that they behave like innate lymphocytes in the following ways: 1) they generally have an activated phenotype and are CD44^{high}; 2) they are selected by hematopoietic cells (14), 3) they can be activated in the periphery by their thymic selecting ligand/agonist; 4) they rapidly produce cytokines upon activation (15); and 5) their gene expression phenotype has special features differing from that of adaptive T cells (16).

Recently, we have described a peripheral subset of MHC class Ib-restricted CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells that control experimental autoimmune encephalomyelitis (EAE)[4] by killing disease-mediating CD4$^+$ T cells in an Ag-specific manner (11, 17, 18). These T cells appear to have similarities with the gut-residing CD8$\alpha\alpha^+$ TCR$\alpha\beta^+$ intraepithelial lymphocyte population in that they both express the CD8$\alpha\alpha^+$ homodimer that can bind TL tetramers (18, 19). Class Ib MHC molecules appear to play an important role in the development or survival of CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ intraepithelial lymphocytes; these cells are present in mice lacking class Ia MHC and CD1 molecules (20–23). Reconstitution of murine SCID recipients with thymus-derived, self-reactive intraintestinal CD8$\alpha\alpha^+$ TCR$\alpha\beta^+$ T cells prevents colitis disease (24). These studies indicate that a population of class Ib, MHC-restricted CD8$\alpha\alpha^+$ TCR$\alpha\beta^+$ T cells play an important role in controlling autoimmune responses. Consistent with the role of Qa-1-restricted CD8$\alpha\alpha^+$ TCR$\alpha\beta^+$ T cells in immune regulation, Qa-1 dependence for the induction of CD8$^+$ regulatory T cells (Tregs) has been shown in T cell vaccination studies as well as in Qa-1-deficient mice (12, 13). Additionally, we have shown that a peptide from the conserved region of the TCR binds to Qa-1 molecules and is presented to CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells (11). It is clear from earlier studies that Qa-1 can present both foreign (*Salmonella* GroEL) and self-Ags (insulin, heat-shock protein 60, and TCR peptide) to CD8$^+$ T cells (6, 11, 25, 26). However, a detailed mechanism for the presentation of self-peptides in the Qa-1 pathway has yet to be described.

*Laboratory of Autoimmunity, Torrey Pines Institute for Molecular Studies, San Diego, CA 92121; and †Imaging Facility, La Jolla Institute for Allergy and Immunology, La Jolla, CA 92037

Received for publication January 28, 2009. Accepted for publication April 2, 2009.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by National Institutes of Health Grant R0I AI052227 and by grants from the Multiple Sclerosis National Research Institute and the Diabetes National Research Group (to V.K.).

[2] Current address: Department of Cancer Immunology and AIDS, Harvard Medical School, Boston, MA 02115.

[3] Address correspondence and reprint requests to Dr. Vipin Kumar, Laboratory of Autoimmunity, Torrey Pines Institute for Molecular Studies, 3550 General Atomics Court, San Diego, CA 92121. E-mail address: vkumar@tpims.org

[4] Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; Ac, acetylated; DC, dendritic cell; ER, endoplasmic reticulum; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; poly(I:C), polyinosinic:polycytidylic acid; PTx, pertussis toxin; Treg, regulatory T cell.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.0900316

Dendritic cells (DCs) are considered the most potent and versatile APCs, capable of efficiently acquiring Ag in the periphery then trafficking to draining lymph nodes where they can interact with and induce optimal activation of effector function in T cells (27). Furthermore, DCs regulate T cell tolerance in both the thymus and periphery (28, 29). It has been shown that DCs can capture apoptotic cells and process and present proteins derived from these cells to both $CD4^+$ and $CD8^+$ T cells (30–32). Studies have suggested that, under noninflammatory conditions, tolerance results due to incomplete signaling at the synapse between the DC and the T cell (33, 34). However, within an inflammatory milieu DCs receive signals (e.g., TLR agonist) to become fully activated to mediate productive T cell responses (27). We proposed earlier that DCs capture disease-mediating $CD4^+$ T cells that are undergoing cell death and present peptides derived from the TCR in the context of Qa-1 to CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells (17, 35). Consistently, interactions between DCs and Qa-1 have been shown to play an important role in the induction of $CD8^+$ T cell-mediated immune regulation (36).

In the current study we have investigated the Qa-1 presentation pathway by which a self-Ag derived from an apoptotic $CD4^+$ T cell is able to cross-prime an immunoregulatory CD8$\alpha\alpha^+$ TCR$\alpha\beta^+$ T cell response. We have recently characterized several Qa-1a-restricted, TCR p42–50 peptide-reactive CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell clones and lines (11, 17, 18). These cells are physiologically primed and are involved in recovery from EAE induced by the myelin basic protein (MBP) acetylated N-terminal peptide Ac1–9, designated throughout as MBP(Ac1–9). Using well-characterized Qa-1a-restricted CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell lines and clones (11, 18) as our responder T cell population, we demonstrate that bone marrow-derived DCs can capture apoptotic Vβ8.2$^+$ T cells and process TCR-derived peptide Ags by a class Ib MHC pathway in vitro. We show that the priming ability of DCs can be enhanced after treatment with TLR agonists or blocked after treatment with antagonists of endosomal and proteasomal Ag processing. We demonstrate CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell-priming and protection from EAE by DCs loaded with Vβ8.2$^+$ apoptotic T cells. These studies are the first to reveal a physiologically relevant mechanism for the activation of CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell-mediated immune regulation. Furthermore, they delineate a novel immunotherapeutic protocol that exploits the immunostimulatory capacity of DCs in the delivery of self-Ags for effective immune regulation.

Materials and Methods

Mice

B10.PL, PL/J, and C57BL/6 (BL/6) mice were purchased from The Jackson Laboratory. Mice were kept under pathogen-free conditions in our own colony at the Torrey Pines Institute for Molecular Studies (TPIMS; San Diego, CA). Female mice, age matched at 8–16 wk, were used in the described experiments. Treatment of animals was in compliance with federal and institutional guidelines and approved by the TPIMS Animal Care and Use Committee.

Peptides

The amino acid sequence for MBP(Ac1–9) is AcASQKRPSQR, and that for myelin oligodendrocyte glycoprotein (MOG) peptide 35–55 is MEVGWYRSPFSRVVHLYRNGK. Amino acid sequences for TCR CDR2 region peptides from the Vβ8.2 chain are GLRLIHYSY for p42(42–50) and PSQENFSLI for p69(69–77).

Induction of EAE and clinical evaluation

For induction of EAE, H-2^u mice, (PL/J or B10.PL) and (B10.PL × BL/6)F_1 were immunized s.c. with 150 μg of MBP(Ac1–9) or 100 μg of MOG emulsified in CFA, respectively, and i.p. with 0.15 μg of pertussis toxin (PTx; List Biological Laboratories) in PBS. Forty-eight hours later, mice were injected with 0.15 μg of PTx in PBS. Mice were observed daily for the clinical appearance of EAE. Disease severity was scored on a five-point scale (37): 1, flaccid tail; 2, hind limb weakness; 3, hind limb paralysis; 4, whole body paralysis; and 5, death.

DC generation

Murine DCs were derived from tibias and femurs by flushing out the bone marrow with RPMI 1640 medium (38). RBCs were lysed and bone marrow was cultured in 24-well plates at 1×10^6 cells/ml in complete RPMI 1640 medium containing 10 ng/ml IL-4 and 25 ng/ml GM-CSF (or, for type 1 DC, 20 ng/ml IL-3, IL-12, and GM-CSF) for 5–7 days. The medium was refreshed on days 3 and 5. In all of the experiments, including adoptive transfers, syngeneic DCs were used. For some experiments DCs were fixed by suspending the cells at 2×10^6/ml in PBS containing 0.05% glutaraldehyde for 30 s at 37°C. Lysine (0.2 M) was added to stop the reaction. Recombinant cytokines were purchased from Peprotech.

ELISA

IFN-γ levels in the supernatants from T cell assays were measured by a sandwich ELISA. Briefly, 96-well Maxisorp F96 plates (Nalge Nunc) were coated with anti-IFN-γ capture Ab overnight at 4°C. Plates were blocked with PBS containing 10% FBS before 50 μl of supernatants were added and incubated overnight at 4°C. After extensive washing, plates were incubated with a biotinylated anti-IFN-γ detection Ab. Plates were developed using avidin-peroxidase and 2–2′-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) substrate (Sigma-Aldrich). OD_{405} was measured and cytokine levels were determined against a recombinant protein standard. All Abs were purchased from BD Pharmingen.

ELISPOT

IFN-γ-producing cells were enumerated from splenocyte populations isolated from immunized mice by cellular ELISPOT assay (39). Briefly, splenocytes (5×10^6 cells/ml) were cultured for 48 h in 24-well plates either with p42, p69 (40 μg/ml), or medium alone. Millititer HA nitrocellulose plates (Millipore) were coated overnight at 4°C with anti-IFN-γ Abs. After blocking coated plates, Ag-stimulated cells were added at graded concentrations for 24 h at 37°C. The wells were then incubated with biotin-conjugated anti-IFN-γ mAbs followed by incubation with avidin peroxidase (Vector Laboratories). Spots were developed by the addition of 3-amino-9-ethylcarbazole substrate (Sigma-Aldrich) and counted using a computerized image analysis system (Lightools Research) and the image analyzer program NIH Image 1.61.

In vitro T cell stimulation assays

Immature bone marrow-derived DCs (1×10^6) were pulsed with 1×10^6 apoptotic or untreated T cells, peptide (20 μg/ml), or PBS for 8–12 h, In most experiments DCs were treated with activation modulators (for example, LPS; Sigma-Aldrich) for 4–12 h and enriched by positive selection using anti-CD11c microbeads (Miltenyi). $CD11c^+$ DCs were disabled (irradiated or glutaraldehyde-fixed) and incubated along with 2D11 T responder cells (2×10^4/well) for the duration of the assay at 37°C in 5% CO_2. Supernatants were harvested and collected at 48 h for IFN-γ secretion analysis.

For experiments analyzing the effect of the Ag processing/presentation blockade, the following titrated inhibitors were first added to DCs for a duration of 2 h: lactacystin (50 μM), concanamycin A (50 nM), monensin (5 μM), or brefeldin A (1–5 μg/ml); cycloheximide (10–100 μg/ml) was added for a duration of 5 h. DCs were then washed and pulsed with peptide or apoptotic T cells for a total of 8 h (the final 4 h in the presence of LPS at 1 μg/ml). DCs were positively selected using anti-CD11c microbeads (Miltenyi Biotec) and fixed with glutaraldehyde before coculture with responder T cells. For IFN-γ secretion analysis, supernatants were harvested at 48 h. All inhibitors were purchased from Sigma-Aldrich except for cycloheximide and lactacystin, which were purchased from A.G. Scientific.

Cytotoxicity assay

Cytotoxicity mediated by T cells was tested using a standard 4-h ^{51}Cr-release assay with minor modifications. Target cells (Con A-activated blasts) were labeled with ^{51}Cr (MP Biomedicals) at 37°C for 45 min (in complete DMEM medium). A total of 10,000/well ^{51}Cr-labeled targets and 200 ng/well peptides were added to round-bottom, 96-well plates. Plates were then incubated at 37°C with 10% CO_2 for 1 h. Effector cells (splenocytes from immunized mice or the CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell line XT-14; Ref. 11) were added in triplicate to the wells at the indicated E:T cell ratios to a total volume of 200 μl. The plates were incubated for 4 h before 50 μl of supernatant was transferred into sample plates. A total of 150 μl/well scintillation liquid (OptiPhase SuperMix; PerkinElmer) was added, and the

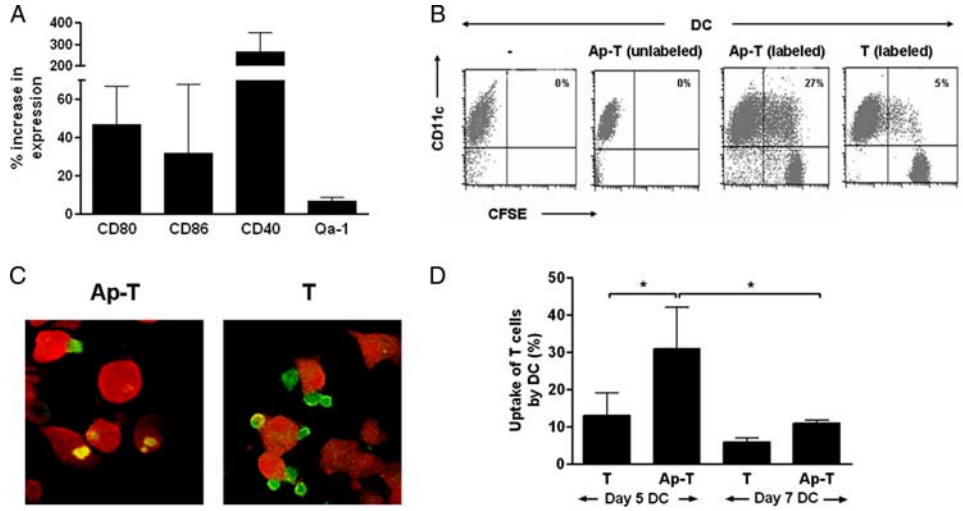

FIGURE 1. Ingestion of apoptotic T cells by DCs. *A*, Percentage increase of the expression of cell surface markers on day 7 vs day 5 culture DCs as determined by flow cytometry. *B*, Nontreated (T) or anti-FAS-treated apoptotic (Ap-T) Vβ8.2$^+$ T cells (2×10^6) were labeled with CFSE dye (5 μM) and cultured at 37°C for 12 h in the presence of PE-labeled syngeneic CD11c$^+$ DCs (2×10^6). Colocalization of CD11c$^+$ cells with CFSE$^+$ cells was analyzed by flow cytometry. *C*, Confocal analysis of SNARF-1 (10 μM)-labeled DCs (2×10^5) cultured with CFSE-stained Ap-T (*left panel*) or T (*right panel*) cells (5×10^5). Original magnification of ×40 is shown. *D*, Uptake of T cells by day 5 or day 7 culture DCs. Data are expressed as the mean percentage (±SD) of the DC population that has colocalized with CFSE$^+$ T cells. *, $p < 0.05$. Data are representative of 3–5 independent experiments.

sample plates were read on a Trilux scintillation gamma counter. Spontaneous ^{51}Cr-release was measured in control wells containing target cells with medium alone. Maximum release values were obtained by lysis of target cells with 2.5% Triton X-100 (Sigma-Aldrich). The percentage of specific release was calculated as follows: (experimental release − spontaneous release)/(maximal release − spontaneous release) × 100.

Flow cytometry

T cell apoptosis induction. Forty-eight hours after in vitro activation with their cognate Ag, the desired number of CD4$^+$ T cells (either a MBP(Ac1–9)-reactive Vβ8.2$^+$CD4$^+$ T cell clone (100% Vβ8.2TCR$^+$) or a control Vβ8.2-CD4$^+$ T cell clone (100% Vβ14TCR$^+$), both derived from H-2^u mice (37), were incubated in complete RPMI 1640 medium overnight at 37°C in 5% CO_2 in the presence of 5 μg/ml anti-Fas Ab (BD Pharmingen). To determine apoptosis induction, 1×10^5 T cells in 100 μl of buffer were stained with 10 μl/ml annexin V (BD Pharmingen). Using flow cytometry, apoptosis induction was confirmed using two parameters: 1) a counterclockwise shift of the T cell population in the forward vs side scatter dot plot; and 2) a significant right shift of the peak on the FL1 (fluorescence channel 1) histogram axis indicating annexin V staining.

DC capture of apoptotic T cells. Thoroughly washed CFSE (Molecular Probes)-labeled (5 μM) apoptotic or nonapoptotic T cells ($1–5 \times 10^6$ cells) were directly added to day 5–7 DC cultures in 24-well plates. After 12 h of culture (37°C in 5% CO_2), cells were harvested and stained with PE-labeled anti-CD11c Ab. Colocalization of CD11c$^+$ DCs with CFSE$^+$ apoptotic T cells was analyzed by flow cytometry.

Characterization of the maturation state of a DC

The effect of chemical and biological DC modulators was determined by analyzing the levels of cell surface markers. Briefly, day 5–7 DC cultures were incubated with various concentrations of the chosen modulator and control. DCs were harvested and stained with anti-CD11c (PE) in addition to FITC-labeled anti-CD40, CD80, CD86, MHC-class II, or matched isotype control Abs. To determine the effects on Qa-1 expression, BL/6-derived DCs were stained with biotin-labeled anti-Qa-1b Ab before staining with anti-CD11c (PE) and streptavidin (FITC) Abs. All Abs were purchased from BD Pharmingen.

Confocal analysis

In Lab-Tek II chambered coverglass slides (Nalge Nunc), CFSE-labeled (5 μM) apoptotic or untreated T cells (0.8×10^6 cells) were mixed with day 5 DCs (0.5×10^6) stained with 10 μM SNARF-1 (Molecular Probes), in RPMI 1640 complete medium supplemented with IL-4 (10 ng/ml) and GM-CSF (25 ng/ml) for 12 h at 37°C in 5% CO_2. Supernatants were carefully removed. Cells were fixed to the slide by adding 100 μl of 2% paraformaldehyde per chamber for 30 min at room temperature in the dark. Cells were visualized on a Bio-Rad MRC-1024 confocal microscope equipped with a ×40/1.3 numerical aperture, oil immersion Nikon objective. Images were recorded with Laser Sharp 2000 for Windows NT. Analysis was performed using the ImageJ (merge; ZProjection) program.

Real-time PCR

Total mRNA was extracted from splenocytes using the RNeasy mini kit (Qiagen) and subjected to cDNA synthesis with an oligo(dT)12–18 primer. Real-time PCR to detect expansion of CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell clones was performed as previously described using the Brilliant SYBR Green quantitative PCR kit (Stratagene) on a Stratagene Mx3000p machine (18).

Statistical analysis

Data are expressed as mean ± SD for each group. Statistical differences between groups were evaluated using a Mann-Whitney *U* test using GraphPad Prism 4.03 software. Values of $p < 0.05$ were considered statistically significant.

Results

Dendritic cells ingest Vβ8.2$^+$CD4$^+$ T cells undergoing apoptosis

To determine the ability of DCs to ingest apoptotic T cells, immature and mature syngeneic bone marrow-derived CD11c$^+$ DCs from H-2^u mice were used. Throughout these studies, MBP Ac1–9-reactive, Vβ8.2$^+$CD4$^+$ T cell clones generated from Vβ8.2$^+$ TCR-transgenic B10.PL (H-2^u) mice were used as a source of apoptotic T cells (40). These cells were 100% positive for the Vβ8.2$^+$ TCR as confirmed by flow cytometry. Day 5 DCs were determined to be in an immature state based upon the level of cell surface expression of costimulatory and MHC molecules. In comparison, day 7 DCs were considered to be of a mature phenotype as indicated by the up-regulation of their costimulatory molecules CD40, CD80, and CD86 (Fig. 1*A*). Notably, no significant difference in the expression of the Qa-1 molecule between day 5 and day 7 DCs was detected. Apoptosis was induced in Vβ8.2$^+$CD4$^+$ T cells by UV irradiation or by targeting the FAS pathway using an anti-FAS Ab. Equivalent results throughout this study were obtained using either treatment. Apoptosis was confirmed by annexin V staining and flow cytometry and/or by DNA fragmentation analysis. CD11c PE-stained syngeneic DCs were cocultured with

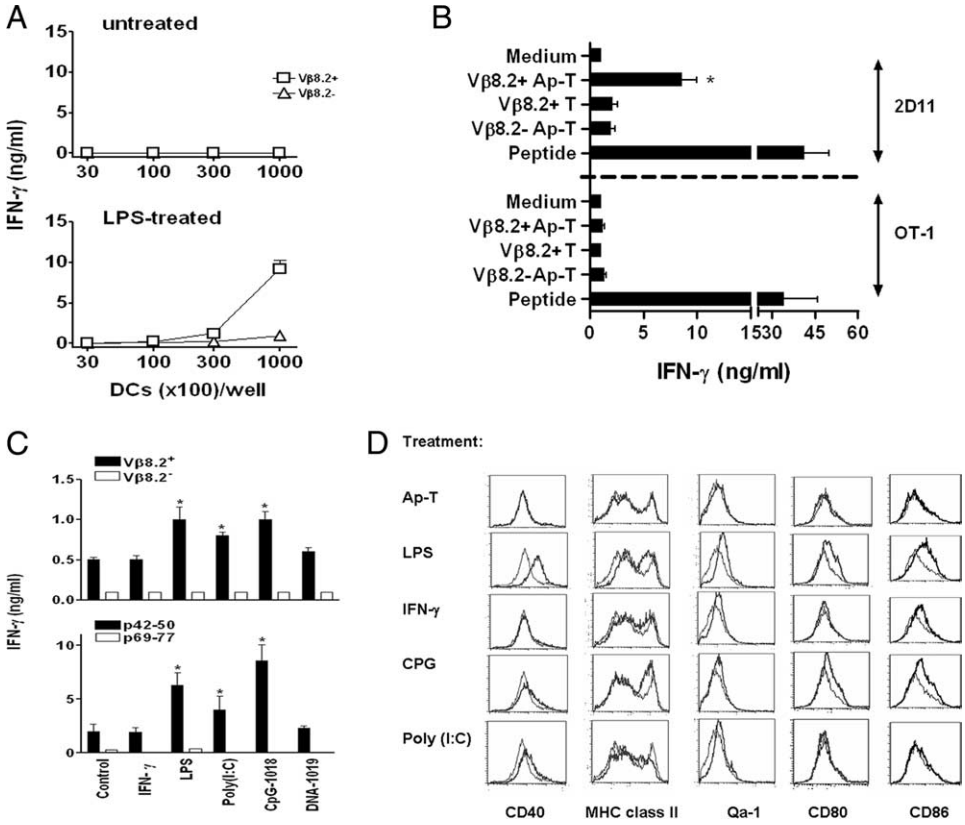

FIGURE 2. Stimulation of Qa-1-restricted CD8αα+TCRαβ+ T cells by TLR agonist-activated DCs pulsed with apoptotic Vβ8.2+ CD4+ T cells (Ap-T). *A*, DCs (1×10^6) were pulsed for 8 h with 1×10^6 Vβ8.2+ or Vβ8.2− Ap-T cells. DCs were separated from noningested Ap-T cells using anti-CD11c microbeads. Purified CD11c+ DCs were then incubated in the presence or absence of LPS (1 μg/ml) for 12 h, washed, and cocultured with the CD8αα+TCRαβ+ T cell clone 2D11 (2×10^4) (11). IFN-γ secretion was measured in 48-h culture supernatants. *B*, A summary of data collected from three independent experiments described in *A*. Additionally, LPS-treated DCs pulsed with peptide, the TCR p42–50 peptide (20 μg/ml) for the CD8αα+TCRαβ+ T cell clone 2D11 and the SIINFEKL peptide (20 μg/ml) for OT-1 CD8αβ+ T cells, were included as positive controls. Values are mean ± SD for IFN-γ secretion. ∗, $p < 0.05$. *C*, DCs pulsed with either apoptotic Vβ8.2+ or Vβ8.2− T cells (*top panel*) or with the cognate p42 or an irrelevant p69 peptide (*bottom panel*) were treated for 4–12 h with IFN-γ (100 ng/ml), LPS (1 μg/ml), poly(I:C) (10 μg/ml), CpG (1 μg/ml), or control DNA (1 μg/ml). DCs (3×10^4) were cocultured with the CD8αα+TCRαβ+ T cell clone 2D11 (2×10^4), and IFN-γ secretion was measured in 48-h culture supernatants. Values are mean ± SD. ∗, $p < 0.05$. *D*, Cell surface expression of activation markers on DCs. Following treatment with the stated agents, CD11c+ cells were gated and analyzed for cell surface markers by flow cytometry. The thick line represents staining after treatment and the thin line represents staining with no treatment. Data are representative of three independent experiments.

CFSE-stained Vβ8.2+CD4+ T cells (Fig. 1*B*). Twenty-seven percent of the DCs became associated with apoptotic T cells after coculture for 12 h. In comparison, only 5% DCs became associated with nonapoptotic T cells. To further examine whether DCs were ingesting the apoptotic T cells, confocal microscopy was used with SNARF-1 (red)-labeled DCs and CFSE (green)-labeled T cells (Fig. 1*C*). Confocal analysis indicated that nonapoptotic T cells remained in close contact with the DC population at the cell surface, whereas apoptotic T cells were engulfed by DCs. Under these experimental conditions, activated CD4+ T cells do not express CD11c as confirmed by flow cytometry (data not shown).

To determine whether the maturation state of DCs influences their ability to uptake apoptotic T cells, we compared DCs cultured for 7 days with those cultured for 5 days. On average, day 5 immature DCs were three times (31 vs 11%) more efficient at taking up apoptotic Vβ8.2+CD4+ T cells than mature DCs (Fig. 1*D*).

DCs pulsed with apoptotic but not nonapoptotic Vβ8.2+ T cells stimulate Qa-1-restricted CD8αα+TCRαβ+ T cells

Previous studies have demonstrated that DCs can phagocytose apoptotic monocytes and B cells and process and present peptides derived from the captured cells in the context of MHC class I and II molecules, respectively (30, 31). However, the ingestion of apoptotic T cells and the cross-presentation of TCR-derived peptide have not been described. Furthermore, the pathways of self-Ag presentation via the MHC class Ib molecule Qa-1 have not been well characterized. To investigate these issues, we determined whether syngeneic DCs pulsed with apoptotic Vβ8.2+CD4+ T cells could stimulate the Qa-1-restricted CD8αα+TCRαβ+ T cell clone 2D11 in vitro. 2D11 T cells have been shown to be Qa-1-restricted and specific for a conserved CDR2 region peptide derived from the TCRVβ8.2 chain (11, 18). 2D11 cells were cocultured with DCs that had been pulsed with apoptotic T cells. Initial coculture experiments with immature day 5 CD11c+ DCs failed to induce detectable IFN-γ secretion or proliferation in 2D11 T cells (Fig. 2*A*, *top panel*). Notably, pulsing day 5 DCs with apoptotic T cells did not induce maturation, suggesting that the cloned CD8αα+TCRαβ+ T cells may not receive optimal stimulatory signals from the immature DCs. Thus, DCs were activated (following pulsing) with the TLR4 agonist LPS. LPS-treated DCs pulsed with apoptotic Vβ8.2+ T cells stimulated the CD8αα+ TCRαβ+ T cell clone 2D11 in vitro as determined by IFN-γ production (Fig. 2*A*, *bottom panel*). Stimulation was dependent on the DCs being pulsed with cells expressing the Vβ8.2+ TCR that were undergoing apoptosis (Fig. 2*B*). In contrast, DCs pulsed with nonapoptotic Vβ8.2+CD4+ T cells or apoptotic Vβ8.2−CD4+ T

cells did not induce significant stimulation of the T cell clone 2D11. To control for non-Ag-specific stimulation, we showed that the OVA peptide-reactive OT-1 T cell clone was not activated by LPS-treated DCs pulsed with Vβ8.2$^+$ T cells (Fig. 2*B*). In summary, activated DCs that have been loaded with Vβ8.2$^+$ apoptotic T cells can stimulate Ag-specific CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells in vitro.

TLR signaling augments DC-mediated stimulation of Qa-1-restricted CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells

CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell stimulation was dependent on the prior activation of DCs with LPS. We determined whether activating DCs enhanced cross-presentation through other innate TLRs and whether enhanced CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell stimulation correlated with cell surface up-regulation of costimulatory and Ag-presenting molecules. Fig. 2*C* shows that the IFN-γ response of the CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell clone 2D11 (11, 17, 18) was augmented (up to 2-fold higher than control) when immature DCs were treated with LPS (TLR4), polyinosinic:polycytidylic acid (poly(I:C); TLR3), or CpG (TLR9), but not when control DNA-1019 or IFN-γ were used. Stimulation of the T cell clone 2D11 by peptide p42–50, which directly binds to cell surface Qa-1 molecules, was also increased when TLR-activators were added to DC preparations (Fig. 2*C*). Flow cytometric analysis showed that DC treatment with TLR receptor agonists was associated with the up-regulation of the cell surface costimulatory molecules CD40, CD80, CD86, and MHC molecules, including the nonclassical class I molecules Qa-1 and MHC class II (Fig. 2*D*). Up-regulation was dependent on the class of agonist used. LPS caused the most significant change in the DC phenotype, and poly(I:C) and IFN-γ caused the least change.

It has been previously reported that ingestion of necrotic but not apoptotic cells can induce DC maturation (34). To determine whether ingested apoptotic Vβ8.2$^+$ cells cause maturation, the cell surface phenotype of the DC was analyzed after coculture with apoptotic T cells. The cell surface levels of the maturation markers CD80, CD86, CD40, and MHC were not significantly affected in these cultures in comparison to the up-regulation observed on LPS-activated DCs (Fig. 2*D*). Thus, immature DCs are proficient at ingesting apoptotic Vβ8.2$^+$ T cells in a manner that does not affect their maturation state as indicated by these markers.

Ag-processing inhibitors suggest an endocytic pathway for class Ib MHC cross-presentation

To determine whether syngeneic DCs were directly presenting antigenic determinants from apoptotic T cells independently of ingestion and intracellular processing, we examined the ability of glutaraldehyde-fixed DCs to stimulate CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells. Fixing LPS-treated DCs before pulsing with apoptotic T cells failed to activate the CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell clone 2D11 (Fig. 3).

Once phagocytosed by the DC, captured material enters an intracellular Ag-processing pathway. While traveling through this pathway, captured material is broken down into smaller protein fragments (epitopes) that can be presented on the cell surface in association with MHC molecules (41). We investigated the effect of inhibitors of Ag processing in an attempt to delineate the pathway for the presentation of exogenous TCR-derived peptides in the context of Qa-1 by DCs. Concanamycin and monensin were used as inhibitors of endosomal activity, and lactacystin was used to block proteasome degradation. Data presented in Fig. 3 clearly show that treatment of DCs with either lactacystin (50 μM), concanamycin A (50 nM), or monensin (5 μM) significantly blocked the cross-presentation (by ~50%; $p < 0.05$). Trypan blue exclusion analysis confirmed that these inhibitors did not cause cell death in the DC population. Flow cytometric analysis revealed no change in cell surface phenotype between the inhibitor and vehicle control-treated DCs with respect to levels of costimulatory and MHC molecules. Furthermore the ability of the DCs to directly present the TCR p42–50 peptide to CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell clone 2D11 was not significantly altered between inhibitor- and vehicle control-treated cells (Fig. 3).

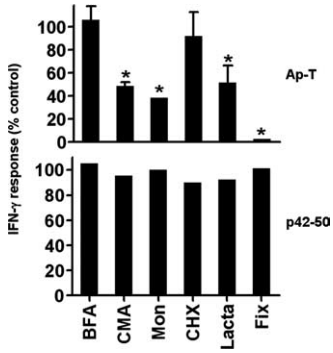

FIGURE 3. Inhibition of DC cross-presentation to Qa-1-restricted CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells by Ag processing and presentation inhibitors. Syngeneic DC cultures were treated with brefeldin A (BFA; 1–5 μg/ml), concanamycin A (CMA; 50 nM), monensin (Mon; 5 μM)), cycloheximide (CHX; 10–100 μg/ml), or lactacystin (Lacta; 50 μM) for a predetermined period of time or were fixed (Fix) with 0.05% glutaraldehyde. DCs were pulsed with Vβ8.2$^+$ apoptotic T cells (Ap-T) or p42 peptide (p42–50) followed by activation with LPS and purification using CD11c microbeads. CD11c$^+$ DCs (3×10^4) were cocultured with the CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell clone 2D11 (2×10^4). IFN-γ secretion was measured in 48-h culture supernatants. Results were expressed as the percentage of the IFN-γ response in comparison to DCs pulsed with Ap-T cells or peptide treated with vehicle control. Vehicle control responses ranged from ~1000–2000 pg/ml IFN-γ. Values are mean ± SD. Data are representative of three independent experiments. *, $p < 0.05$.

Surprisingly, DC treatment with brefeldin A (1–5 μg/ml), the inhibitor of MHC egress out of the endoplasmic reticulum (ER), or with cycloheximide (10–100 μg/ml), the protein synthesis inhibitor, had no significant effect on the stimulation of the CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell clone 2D11. This suggests that the TCR peptides are not processed through the ER and that new protein synthesis of Qa-1 molecules is not required. These results support a pathway of cross-priming that includes endosomal and cytosolic Ag processing independent of ER compartment use.

Intraperitoneal injection of Vβ8.2$^+$ apoptotic T cell-pulsed DCs primes Qa-1-restricted CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells

The ability of DCs loaded with apoptotic Vβ8.2$^+$ T cells to prime the CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell clone 2D11 in vivo was investigated. Immature syngeneic DCs were either unpulsed, Vβ8.2TCR p42–50 peptide pulsed, or apoptotic Vβ8.2$^+$ T cell pulsed. DCs were purified using anti-CD11c microbeads and activated with LPS before i.p. injection (1×10^6 DCs/mouse). On day 5 splenocytes were harvested. The frequency of the p42–50 peptide-reactive T cell Ag recall response was determined by IFN-γ ELISPOT. Fig. 4*A* shows that splenocytes from PL/J mice immunized with DCs pulsed with Vβ8.2$^+$ apoptotic T cells or peptide p42–50, demonstrated a significantly higher ($p < 0.05$) recall response to p42–50 than mice injected with unpulsed DCs. Additional experiments performed using splenocytes isolated for CD8α expression confirmed that recall responses to p42–50 peptide resided within the CD8α^+ T cell population (data not shown). Note that in the CD8α isolation experiments, irradiated whole splenocyte populations were used as APCs to ensure that CD8α^+ DCs were available in the cultures. In summary the above results indicate that adoptive

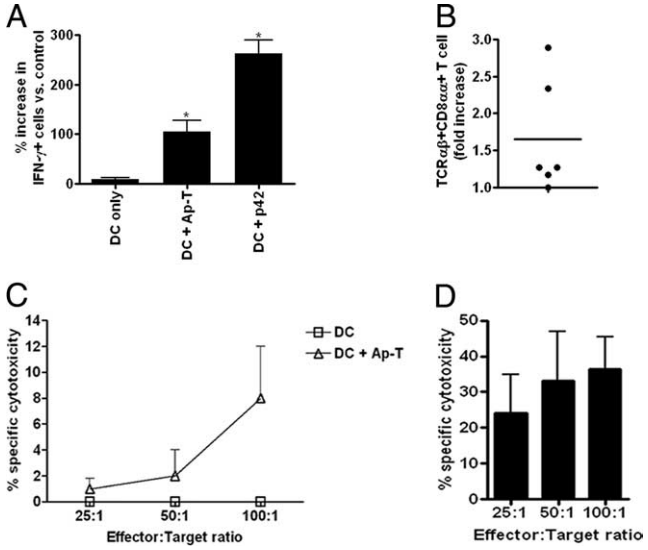

FIGURE 4. DCs pulsed with apoptotic T cells (Ap-T) prime class Ib-restricted CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells in vivo. *A*, LPS-activated syngeneic CD11c$^+$ DCs (1×10^6) pulsed with Vβ8.2$^+$ Ap-T cells (DC + Ap-T), p42–50 peptide (DC + p42), or not pulsed (DC only) were purified and injected i.p into naive syngeneic mice. Five days following injection, spleens were harvested and the frequency of IFN-γ^+ cells was determined in an ELISPOT assay after in vitro recall challenge to TCR p42–50 peptide or a control p69–77 peptide. Results are displayed as the percentage change in the number of IFN-γ^+ cells between p42–50 and the control peptide for each treatment. The control peptide response was between 250 and 500 IFN-γ spots per 10^6 spleen cells; $*$, $p < 0.05$. *B*, Primers specific for the CDR3 region of the dominant CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell clone 2D11 were used to detect amplification of this T cell population by real-time PCR after the transfer of DCs derived from Vβ8.2$^+$ Ap-T- or PBS-immunized syngeneic mice. Expression of CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell clone 2D11-specific transcripts are presented as an expansion fold increase after the transfer of DCs from Ap-T- vs PBS-immunized mice. Six mice in each group were analyzed. *C*, LPS-activated DCs (1×10^6) pulsed with Vβ8.2$^+$ Ap-T cells or left unpulsed were injected i.p on day 0 and day 7 into naive syngeneic mice. A ^{51}Cr-cytotoxicity assay was performed on day 14 with splenocyte effectors and blast cell targets pulsed with either p42–50 or an irrelevant MBP peptide. Specific cytotoxicity is calculated as follows: (experimental release − spontaneous release)/(maximal release − spontaneous release) × 100%. *D*, The p42–50 peptide-reactive CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell line XT-14 (11) was used as a positive control. Values are mean ± SD. Data are representative of four independent experiments.

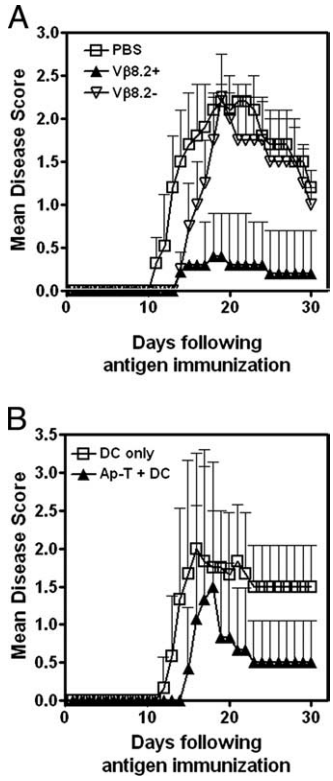

FIGURE 5. Attenuation of EAE by injection of DCs pulsed with Vβ8.2$^+$ apoptotic T cells (Ap-T). *A*, Groups of H-2^u mice were injected i.p with 5×10^6 syngeneic apoptotic Vβ8.2$^+$ or Vβ8.2$^-$ T cells or PBS on day −7. On day 0 mice were immunized s.c with 150 μg of MBP(Ac1–9) in CFA and PTx. *B*, LPS-activated CD11c$^+$ type 1 DCs pulsed with Vβ8.2$^+$ Ap-T cells (Ap-T + DC) or not pulsed (DC only) were purified and injected (0.8×10^6 cells) i.p into naive (B10.PL × BL/6 crossovers)F$_1$ mice on day −7 and day 1. On day 0 mice were immunized for EAE s.c. with 100 μg of MOG in CFA and PTx. All mice were monitored up to day 30 for symptoms of EAE (as described in *Materials and Methods*). At least six mice in each treatment group are included.

transfer of DCs carrying Vβ8.2$^+$ apoptotic T cells can prime CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell responses in vivo.

Real-time PCR analysis was performed to detect the in vivo expansion of CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells following the transfer of DCs derived from Vβ8.2$^+$ apoptotic, T cell-immunized mice. Splenocytes were harvested 20 days following i.v. transfer of 1.5×10^6 DCs and stimulated for 48 h in vitro with p42–50 peptide before the total mRNA was isolated. Real-time PCR was performed on the cDNA (synthesized from the mRNA) using primers specific for the CDR3 region of the dominant CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell clone (2D11) (18). Fig. 4*B* shows a mean amplification of cDNA specific for the regulatory CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell clones at 1.75 times that of the control group. These data demonstrate the priming of CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells following cross-presentation in vivo.

Next, we determined whether the adoptive transfer of DCs carrying Vβ8.2$^+$ apoptotic T cells could prime p42–50 peptide-reactive CD8$^+$ T cells that displayed cytotoxicity toward activated Vβ8.2$^+$ T cells. On day 0 and day 7 PL/J mice were i.p. injected with LPS-activated DCs that had been pulsed with Vβ8.2$^+$ apoptotic T cells or unpulsed. On day 14 splenocytes were harvested and cytotoxic function was analyzed in ^{51}Cr-release assays. Fig. 4*C* shows that splenocytes isolated from mice immunized with apoptotic Vβ8.2$^+$ T cell-pulsed DCs induced significantly higher death in the target p42–5 peptide blast cells (~8% at the highest E:T ratio) in comparison to splenocytes isolated from DC only-treated mice (0% at highest E:T ratio). XT-14 (a p42–50 peptide-reactive cytotoxic CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell line; Ref. 11) was used as a positive control (Fig. 4*D*). Collectively, these data demonstrate in vivo priming of TCR p42–50 peptide-reactive CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells by DCs that have captured apoptotic Vβ8.2$^+$ T cells.

Attenuation of EAE by injection of apoptotic Vβ8.2$^+$ T cells or DCs pulsed with apoptotic Vβ8.2$^+$ T cells

We determined whether apoptotic T cell- and DC-mediated priming of Qa-1-restricted p42–50 peptide-reactive CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells in vivo could lead to the regulation of Ag-induced EAE. Groups of mice were injected i.p with 5×10^6 apoptotic syngeneic Vβ8.2$^+$ or Vβ8.2$^-$ CD4$^+$ T cell clones or PBS. On day 7 H-2^u mice were immunized s.c with MBP(Ac1–9) peptide/CFA/PTx for the induction of EAE and monitored daily for the clinical symptoms of disease. Fig. 5*A* shows a significant attenuation ($p < 0.0001$) of disease symptoms in the Vβ8.2$^+$ apoptotic T cell-treated

Table I. *Vaccination with DCs pulsed with apoptotic Vβ8.2$^+$ T cells (Ap-T) leads to attenuation of EAE*

Treatment	Maximal Individual Disease Score	Mean Disease Score	Incidence of EAE[a]
PBS	4,3,3	3.3	3/3
DC only	5,4,4,4,4,3,3,3,3,2,0	3.2	10/11
Vβ8.2$^+$Ap-T-pulsed DC	4,3,3,3,3,2,1,0,0,0,0	1.7	7/11
TCR peptide (p42)	4,0,0,0	1	1/4

[a] The number of animals with disease/total number of animals immunized.

group compared with the PBS or Vβ8.2$^-$ apoptotic T cell-treated groups. Results demonstrate that protection from EAE disease is associated with the adoptive transfer of apoptotic Vβ8.2$^+$ T cells, but not Vβ8.2$^-$ T cells, into the H-2^u mouse.

A number of studies from our laboratory and others have shown that induction of this immune regulation is only effective under the conditions of a Th1-like cytokine milieu (35, 42–45). Furthermore, other experiments indicated that optimal ex vivo priming of TCR-reactive CD4$^+$ Treg clones and disease protection was associated with DCs isolated from type 1 inflammatory conditions (I. Maricic, F. Ria, S. Schneider, and V. Kumar, submitted for publication). Thus, we generated type 1 DCs by culturing bone marrow-derived cells in the presence of IL-3, IL-12, and GM-CSF for 5 days. We vaccinated mice with LPS-activated type 1 DCs pulsed with Vβ8.2$^+$ apoptotic T cells. As shown in Fig. 5*B*, vaccination with DCs pulsed with Vβ8.2$^+$ apoptotic T cells resulted in significant attenuation of EAE disease compared with DC only vaccination ($p = 0.0013$). Table I shows maximal disease scores and disease incidence associated with various treatments. As positive and negative controls, mice were vaccinated with the TCR p42–50 peptide (20 μg/mouse) or with PBS, respectively. There was a significant attenuation ($p < 0.05$) in the mean maximal disease score and a lower frequency of disease incidence in animals vaccinated with DCs pulsed with Vβ8.2$^+$ apoptotic T cells in comparison to mice injected with DCs alone.

Discussion

The studies presented here delineate a novel mechanism by which Ags derived from apoptotic CD4$^+$ T cells are presented by DCs in the context of the class Ib MHC molecule Qa-1 to CD8αα$^+$ TCRαβ$^+$ T cells. We show that immature DCs are efficient in the ingestion of apoptotic CD4$^+$ T cells, and upon activation by TLR agonists they are able to stimulate Qa-1-restricted CD8αα$^+$ TCRαβ$^+$ T cell clones in vitro as well as prime them in vivo. We demonstrate a negative feedback mechanism by which Ag receptors derived from disease-mediating CD4$^+$ T cells are cross-presented to a peripheral population of Qa-1-restricted CD8αα$^+$ TCRαβ$^+$ T cells, which results in the suppression of inflammatory disease.

Several cross-presentation pathways for cellular Ags may exist (46), but whether class Ia and class Ib MHC molecules share these pathways is unknown. Our data support a cross-presentation pathway that includes endosomal and proteasomal processing but is independent of trafficking through the ER. Treatment with the ER egress inhibitor brefeldin A and the protein synthesis inhibitor cycloheximide had no significant effect on the ability of DCs to stimulate CD8αα$^+$TCRαβ$^+$ T cells (Fig. 3). These data clearly support a pathway independent of ER trafficking and the de novo synthesis of Qa-1a molecules. Inhibition of cross-presentation by concanamycin A, monensin, or lactacystin suggests that the processing of the TCR β-chain protein from the endocytosed apoptotic T cell follows the endosomal Ag trafficking pathway into the cytosol for proteasomal degradation. The precise compartment in which the peptides become associated with Qa-1a molecules has yet to be determined. Other investigations have suggested that peptides re-enter either endosomal or phagosomal compartments that have recruited ER-associated Ag-processing machinery (e.g., TAP, calnexin, calreticulin, and Sec61) (47–49). They report that shortly after their formation, phagosomes fuse with the ER and acquire Ag-processing machinery components from the ER membrane (47, 48). This specialized phagosome (containing ER components) would be self-sufficient for Ag processing and cross-presentation of particulate exogenous Ag. Retrotranslocation machinery such as Sec61 would transfer protein out of the specialized phagosome into the cytosol for proteasomal processing. These processed peptides would then be transported back into the lumen via TAP and loaded onto MHC class I molecules. However, the existence of these phagosomes/endosomes is controversial, and it was recently reported that a physical continuity between the ER and the phagosome does not occur during phagocytosis (50). Based on our data, we propose a model (Fig. 6) that depicts a pathway through which Ags derived from endocytosed apoptotic T cells are processed and presented by the Qa-1 molecule.

As stated above, our data indicate that Ags derived from the TCR β-chain protein undergo endosomal and protease processing before being loaded onto Qa-1a molecules. This is in partial agreement with an earlier report suggesting that the presentation of soluble insulin by Qa-1b can be blocked using chemical inhibitors of the endosomal processing pathway (51). Although our studies deal with Ag presentation by Qa-1a molecules, most other studies have

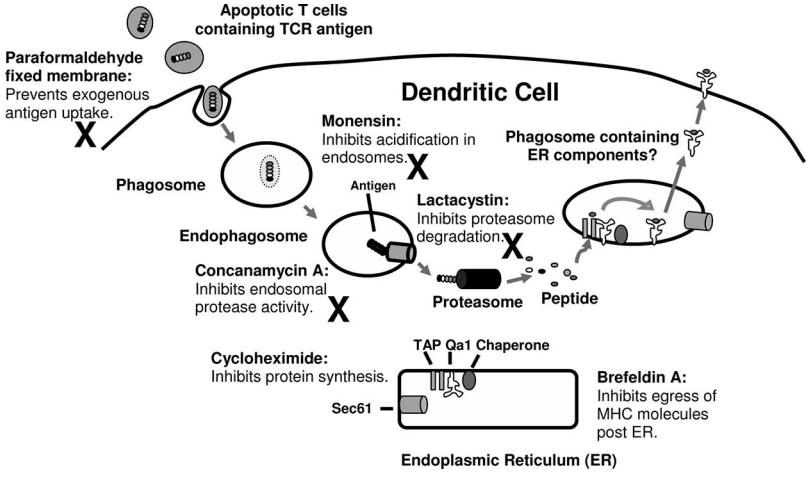

FIGURE 6. A model depicting the DC cross-presentation pathway of Qa-1-associated Ags derived form apoptotic T cells. Apoptotic cell Ags ingested by the DCs are enclosed in endosomal compartments and subjected to an increasingly acidic environment that leads to proteolysis degradation. Monensin and concanamycin A inhibit this process. Ags are transported out of the endosomes into the cytosol for proteasomal processing. Lactacystin can inhibit proteasomal degradation. Rather than trafficking through the endoplasmic reticulum, data predict that the peptide fragments are loaded onto Qa-1 molecules in another compartment, possibly re-entering a phagosomal or endosomal compartment containing Qa-1-loading machinery before being presented on the cell surface. Note that inhibitors blocking this pathway are highlighted with an "X." See *Discussion* for further details.

been performed on the Qa-1b allelic molecules (9, 51, 52). However, due to the structural homology between the two alleles it is unlikely that the presentation pathway exploited by Qa-1a and Qa-1b molecules would be significantly different. Earlier studies suggested an interesting difference between endogenous and exogenous peptide presentation by Qa-1: the dominant Qa-1-binding peptide Qdm (derived from the leader sequence of MHC class I molecules) and the intracellular pathogen *L. monocytogenes* were TAP dependent, whereas the exogenous insulin peptide was TAP independent (52, 53). It was suggested that TAP deficiency may lead to the enhanced presentation of peptides that are usually not displayed in the context of Qa-1 molecules due to competition with the high affinity Qdm peptide (9). We are currently investigating the role of TAP molecules in the presentation of TCR-derived peptides.

The Qa-1a-restricted CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells kill activated Vβ8.2$^+$CD4$^+$ target cells (11, 54). Consistent with this, we have shown that vaccination with DCs pulsed with apoptotic Vβ8.2$^+$ CD4$^+$ T cells can induce a CD8$^+$ cytotoxic T cell response against the target TCR p42 peptide-pulsed blast cells (Fig. 4*C*). Previous experiments indicated that CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells kill only activated Vβ8.2$^+$ T cells, not resting or non-Vβ8.2$^+$ T cells (11, 12). This relates to the appropriate display of relevant Vβ8.2-derived peptide/Qa-1 complexes on the surface of the activated target CD4$^+$ T cells that is dependent on the processing and presentation of recycling TCR Vβ proteins. Qa-1 is up-regulated upon activation and is expressed at low levels on naive/resting T cells (55). Additionally, activation of the target T cell may cause the TCRVβ protein to enter the lysosomal degradation pathway (56) or the proteasomal pathway for presentation on the T cell surface. Enhancement of costimulatory molecules on the Vβ8.2$^+$CD4$^+$ target cells may also facilitate recognition by the CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ Treg. This is supported by a recent report by Yang et al. who showed that autoreactive Th1 cells up-regulated CD80 and CD86 costimulatory molecules on their cell surface upon activation and that blockade of the B7 molecules attenuated the efficacy of T cell vaccination when using these cells in an EAE model (57).

It is noteworthy that Qa1-restricted CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell stimulation is absolutely dependent on the maturation state of the DC (Fig. 2). DCs that have captured apoptotic cells have been shown to mediate T cell tolerance and not productive immune responses (58). Tolerance can be the result of incomplete costimulation provided by the DC to the cognate T cell, leading to anergy or the induction of the CD4$^+$FOXP3$^+$ Treg (59). In this study we found that DCs pulsed with apoptotic Vβ8.2$^+$ T cells could efficiently stimulate CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell responses only after activation of the DC with TLR-agonists, e.g., LPS (Fig. 2). Additionally, we found that pulsing DCs with apoptotic T cells did not affect their maturation state as determined by the up-regulation of cell surface molecules (Fig. 2*D*). We predict that in the steady state, where DCs do not receive activation signals, the CD8$\alpha\alpha^+$ TCR$\alpha\beta^+$ T cell will remain inactivated or tolerized. However, in an inflammatory milieu containing DC-activating molecules, T cell tolerance can be broken and it will become primed to perform its effector function. Tolerization of the Treg population could explain why passively transferred EAE (without adjuvant) results in a severe chronic relapsing course, whereas actively induced EAE (with adjuvant) results in a monophasic disease. Accordingly, protection from EAE in the H-2^u mouse was observed following vaccination with TCR peptides emulsified with inflammatory modulators in IFA or CFA (11, 60). Furthermore, our previous experiments have shown low level priming of a TCR-reactive CD4$^+$ Treg clone by splenocyte-derived DCs from naive H-2^u mice, but priming was augmented when DCs were derived from a mouse during active EAE (T. R. F. Smith, I. Maricic, F. Ria, S. Schneider, and V. Kumar, submitted for publication). Consistently, Liu and colleagues observed that DCs capturing OVA-loaded apoptotic cells in a steady state environment tolerized CD8$^+$ T cells (58). However, when OVA-loaded apoptotic cells were administered together with the agonistic anti-CD40 Ab to mature DCs, the Ag-reactive CD8$^+$ T cells were primed. Propato and colleagues demonstrated that CD40 expression on the apoptotic cell was needed for DC maturation and CTL cross-priming (61). We believe that the CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell response is under stringent control mechanisms and dependent on a level of "help" from the APC and CD4$^+$ Th cells (62).

The priming of cytotoxic CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells following an oligoclonal expansion of Vβ8.2$^+$ T cells during an autoimmune episode may suggest that these cells would also be primed during other inflammatory responses associated with oligoclonal expansion of T cells. For example, in the case of the murine lymphocytic choriomeningitis virus response the viral determinant-reactive T cells using a particular TCR Vβ-chain expand rapidly and account for up to 50% of the peripheral T cell repertoire (63). Following the initial antiviral response, CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells may act to safeguard against uncontrolled T cell expansion (35). Thus, we propose that during an oligoclonal CD4$^+$ T cell-mediated inflammatory response where significant apoptotic cell death in the disease-mediating CD4$^+$ T cells occurs at the site of inflammation e.g., CNS (64, 65), apoptotic T cells are captured by the resident immature DC populations. The Ag-loaded DCs would detect inflammatory stimuli as a signal to migrate from the site of inflammation, e.g., the CNS, to the regional lymph nodes where they could cross-prime a TCR peptide-reactive CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cell response (66, 67). Primed CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells would target Qa-1-associated TCR-antigenic determinants on the cell surface of activated autoreactive T cells to suppress the inflammatory response. Thus, in addition to Ag-induced cell death, proapoptotic cell signaling (e.g., Bim), and exhaustion (68, 69), the targeting of T cells expressing high levels of Qa-1 by CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells may play an important role in the contraction of potentially damaging immune responses. Consistent with this hypothesis, Rawson and colleagues demonstrated that in HIV-infected individuals, DCs cross-presented structural proteins derived from apoptotic T cells that stimulated IFN-γ production in CD8$^+$ T cells (70). Furthermore, they found a positive correlation between the levels of autoreactive CTLs and the numbers of CD4$^+$ T cells undergoing apoptosis in the HIV-infected individual's blood. The study predicted that during inflammatory episodes that are associated with chronic HIV infection, autoreactive effector CD8$^+$ T cells would be primed to kill CD4$^+$ T cells. Thus, the negative feedback mechanism would have immunopathological consequences when the pathogen is not cleared and chronic inflammation persists.

DCs represent a viable immunotherapeutic target for the treatment of autoimmunity, cancer, and infectious disease (71). In this study we show that vaccination with a LPS-activated DC pulsed with apoptotic Vβ8.2$^+$CD4$^+$ T cells induces a CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ CTL response against activated Vβ8.2$^+$ CD4$^+$ T cells (Fig. 4) and protects animals from autoimmune disease (Fig. 5). We predict the in vitro loading of DCs with oligoclonal populations of cells will become a successful strategy to induce immune protection against a variety of diseases associated with the expansion of pathogenic oligoclonal T cell populations. Successful immunotherapy may depend on the generation of immunity against a wide variety of disease-associated Ag targets. Loading DCs with oligoclonal populations of apoptotic T cells could be more advantageous than the

use of peptide antigenic determinants, as the apoptotic cell contains the full repertoire of Ags associated with the pathogenic $CD4^+$ T cell response and is thereby capable of engaging a broad spectrum of Tregs in the control of autoimmune disease.

Acknowledgments

We thank Drs. Eyal Raz and Edith Janssen for their generosity in providing TLR agonist reagents and Dr. Randle Ware for critical reading of the manuscript.

Disclosures

The authors have no financial conflict of interest.

References

1. Braud, V. M., D. S. Allan, and A. J. McMichael. 1999. Functions of nonclassical MHC and non-MHC-encoded class I molecules. *Curr. Opin. Immunol.* 11: 100–108.
2. Lindahl, K. F., D. E. Byers, V. M. Dabhi, R. Hovik, E. P. Jones, G. P. Smith, C. R. Wang, H. Xiao, and M. Yoshino. 1997. H2-M3, a full-service class Ib histocompatibility antigen. *Annu. Rev. Immunol.* 15: 851–879.
3. Pamer, E. G., C. R. Wang, L. Flaherty, K. F. Lindahl, and M. J. Bevan. 1992. H2-M3 presents a *Listeria monocytogenes* peptide to cytotoxic T lymphocytes. *Cell* 70: 215–223.
4. Chun, T., N. V. Serbina, D. Nolt, B. Wang, N. M. Chiu, J. L. Flynn, and C. R. Wang. 2001. Induction of M3-restricted cytotoxic T lymphocyte responses by *N*-formylated peptides derived from *Mycobacterium tuberculosis*. *J. Exp. Med.* 193: 1213–1220.
5. Heinzel, A. S., J. E. Grotzke, R. A. Lines, D. A. Lewinsohn, A. L. McNabb, D. N. Streblow, V. M. Braud, H. J. Grieser, J. T. Belisle, and D. M. Lewinsohn. 2002. HLA-E-dependent presentation of Mtb-derived antigen to human $CD8^+$ T cells. *J. Exp. Med.* 196: 1473–1481.
6. Lo, W. F., A. S. Woods, A. DeCloux, R. J. Cotter, E. S. Metcalf, and M. J. Soloski. 2000. Molecular mimicry mediated by MHC class Ib molecules after infection with Gram-negative pathogens. *Nat. Med.* 6: 215–218.
7. Bouwer, H. G., M. S. Seaman, J. Forman, and D. J. Hinrichs. 1997. MHC class Ib-restricted cells contribute to antilisterial immunity: evidence for Qa-1b as a key restricting element for *Listeria*-specific CTLs. *J. Immunol.* 159: 2795–2801.
8. Seliger, B., H. Abken, and S. Ferrone. 2003. HLA-G and MIC expression in tumors and their role in anti-tumor immunity. *Trends Immunol.* 24: 82–87.
9. Van, H. T., S. Laban, D. Koppers-Lalic, J. Koch, C. Precup, P. Asmawidjaja, R. Offringa, and E. J. Wiertz. 2007. The varicellovirus-encoded TAP inhibitor UL49.5 regulates the presentation of CTL epitopes by Qa-1b1. *J. Immunol.* 178: 657–662.
10. Goyos, A., S. Guselnikov, A. S. Chida, L. F. Sniderhan, S. B. Maggirwar, H. Nedelkovska, and J. Robert. 2007. Involvement of nonclassical MHC class Ib molecules in heat shock protein-mediated anti-tumor responses. *Eur. J. Immunol.* 37: 1494–1501.
11. Tang, X., I. Maricic, N. Purohit, B. Bakamjian, L. M. Reed-Loisel, T. Beeston, P. Jensen, and V. Kumar. 2006. Regulation of immunity by a novel population of Qa-1-restricted $CD8\alpha\alpha^+TCR\alpha\beta^+$ T cells. *J. Immunol.* 177: 7645–7655.
12. Jiang, H., H. Kashleva, L. X. Xu, J. Forman, L. Flaherty, B. Pernis, N. S. Braunstein, and L. Chess. 1998. T cell vaccination induces T cell receptor Vβ-specific Qa-1-restricted regulatory $CD8^+$ T cells. *Proc. Natl. Acad. Sci. USA* 95: 4533–4537.
13. Hu, D., K. Ikizawa, L. Lu, M. E. Sanchirico, M. L. Shinohara, and H. Cantor. 2004. Analysis of regulatory CD8 T cells in Qa-1-deficient mice. *Nat. Immunol.* 5: 516–523.
14. Urdahl, K. B., J. C. Sun, and M. J. Bevan. 2002. Positive selection of MHC class Ib-restricted $CD8^+$ T cells on hematopoietic cells. *Nat. Immunol.* 3: 772–779.
15. Kronenberg, M., and L. Gapin. 2002. The unconventional lifestyle of NKT cells. *Nat. Rev. Immunol.* 2: 557–568.
16. Yamagata, T., C. Benoist, and D. Mathis. 2006. A shared gene-expression signature in innate-like lymphocytes. *Immunol. Rev.* 210: 52–66.
17. Smith, T. R., and V. Kumar. 2008. Revival of $CD8^+$ Treg-mediated suppression. *Trends Immunol.* 29: 337–342.
18. Tang, X., I. Maricic, and V. Kumar. 2007. Anti-TCR antibody treatment activates a novel population of nonintestinal $CD8\alpha\alpha^+$ $TCR\alpha\beta^+$ regulatory T cells and prevents experimental autoimmune encephalomyelitis. *J. Immunol.* 178: 6043–6050.
19. Leishman, A. J., O. V. Naidenko, A. Attinger, F. Koning, C. J. Lena, Y. Xiong, H. C. Chang, E. Reinherz, M. Kronenberg, and H. Cheroutre. 2001. T cell responses modulated through interaction between $CD8\alpha\alpha$ and the nonclassical MHC class I molecule, TL. *Science* 294: 1936–1939.
20. Gapin, L., H. Cheroutre, and M. Kronenberg. 1999. Cutting edge: $TCR\alpha\beta^+$ $CD8\alpha\alpha^+$ T cells are found in intestinal intraepithelial lymphocytes of mice that lack classical MHC class I molecules. *J. Immunol.* 163: 4100–4104.
21. Kurepa, Z., J. Su, and J. Forman. 2003. Memory phenotype of $CD8^+$ T cells in MHC class Ia-deficient mice. *J. Immunol.* 170: 5414–5420.
22. Park, S. H., D. Guy-Grand, F. A. Lemonnier, C. R. Wang, A. Bendelac, and B. Jabri. 1999. Selection and expansion of CD8 α/α T cell receptor α/β intestinal intraepithelial lymphocytes in the absence of both classical major histocompatibility complex class I and nonclassical CD1 molecules. *J. Exp. Med.* 190: 885–890.
23. Das, G., and C. A. Janeway, Jr. 1999. Development of CD8α/α and CD8α/β T cells in major histocompatibility complex class I-deficient mice. *J. Exp. Med.* 190: 881–884.
24. Poussier, P., T. Ning, D. Banerjee, and M. Julius. 2002. A unique subset of self-specific intraintestinal T cells maintains gut integrity. *J. Exp. Med.* 195: 1491–1497.
25. Sullivan, B. A., P. Kraj, D. A. Weber, L. Ignatowicz, and P. E. Jensen. 2002. Positive selection of a Qa-1-restricted T cell receptor with specificity for insulin. *Immunity* 17: 95–105.
26. Davies, A., S. Kalb, B. Liang, C. J. Aldrich, F. A. Lemonnier, H. Jiang, R. Cotter, and M. J. Soloski. 2003. A peptide from heat shock protein 60 is the dominant peptide bound to Qa-1 in the absence of the MHC class Ia leader sequence peptide Qdm. *J. Immunol.* 170: 5027–5033.
27. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature* 392: 245–252.
28. Brocker, T., M. Riedinger, and K. Karjalainen. 1997. Targeted expression of major histocompatibility complex (MHC) class II molecules demonstrates that dendritic cells can induce negative but not positive selection of thymocytes in vivo. *J. Exp. Med.* 185: 541–550.
29. Steinman, R. M., and M. C. Nussenzweig. 2002. Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance. *Proc. Natl. Acad. Sci. USA* 99: 351–358.
30. Inaba, K., S. Turley, F. Yamaide, T. Iyoda, K. Mahnke, M. Inaba, M. Pack, M. Subklewe, B. Sauter, D. Sheff, et al. 1998. Efficient presentation of phagocytosed cellular fragments on the major histocompatibility complex class II products of dendritic cells. *J. Exp. Med.* 188: 2163–2173.
31. Albert, M. L., B. Sauter, and N. Bhardwaj. 1998. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. *Nature* 392: 86–89.
32. Nouri-Shirazi, M., and E. Guinet. 2002. Direct and indirect cross-tolerance of alloreactive T cells by dendritic cells retained in the immature stage. *Transplantation* 74: 1035–1044.
33. Gallucci, S., M. Lolkema, and P. Matzinger. 1999. Natural adjuvants: endogenous activators of dendritic cells. *Nat. Med.* 5: 1249–1255.
34. Sauter, B., M. L. Albert, L. Francisco, M. Larsson, S. Somersan, and N. Bhardwaj. 2000. Consequences of cell death: exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. *J. Exp. Med.* 191: 423–434.
35. Kumar, V. 2004. Homeostatic control of immunity by TCR peptide-specific Tregs. *J. Clin. Invest.* 114: 1222–1226.
36. Chen, W., L. Zhang, B. Liang, Y. Saenger, J. Li, L. Chess, and H. Jiang. 2007. Perceiving the avidity of T cell activation can be translated into peripheral T cell regulation. *Proc. Natl. Acad. Sci. USA* 104: 20472–20477.
37. Kumar, V., and E. E. Sercarz. 1993. The involvement of T cell receptor peptide-specific regulatory $CD4^+$ T cells in recovery from antigen-induced autoimmune disease. *J. Exp. Med.* 178: 909–916.
38. Roy, K. C., I. Maricic, A. Khurana, T. R. Smith, R. C. Halder, and V. Kumar. 2008. Involvement of secretory and endosomal compartments in presentation of an exogenous self-glycolipid to type II NKT cells. *J. Immunol.* 180: 2942–2950.
39. Kumar, V., V. Bhardwaj, L. Soares, J. Alexander, A. Sette, and E. Sercarz. 1995. Major histocompatibility complex binding affinity of an antigenic determinant is crucial for the differential secretion of interleukin 4/5 or interferon γ by T cells. *Proc. Natl. Acad. Sci. USA* 92: 9510–9514.
40. Lafaille, J. J., K. Nagashima, M. Katsuki, and S. Tonegawa. 1994. High incidence of spontaneous autoimmune encephalomyelitis in immunodeficient anti-myelin basic protein T cell receptor transgenic mice. *Cell* 78: 399–408.
41. Jensen, P. E. 2007. Recent advances in antigen processing and presentation. *Nat Immunol.* 8: 1041–1048.
42. Waisman, A., P. J. Ruiz, D. L. Hirschberg, A. Gelman, J. R. Oksenberg, S. Brocke, F. Mor, I. R. Cohen, and L. Steinman. 1996. Suppressive vaccination with DNA encoding a variable region gene of the T-cell receptor prevents autoimmune encephalomyelitis and activates Th2 immunity. *Nat. Med.* 2: 899–905.
43. Kumar, V., and E. Sercarz. 1998. Induction or protection from experimental autoimmune encephalomyelitis depends on the cytokine secretion profile of TCR peptide-specific regulatory CD4 T cells. *J. Immunol.* 161: 6585–6591.
44. Braciak, T. A., B. Pedersen, J. Chin, C. Hsiao, E. S. Ward, I. Maricic, A. Jahng, F. L. Graham, J. Gauldie, E. E. Sercarz, and V. Kumar. 2003. Protection against experimental autoimmune encephalomyelitis generated by a recombinant adenovirus vector expressing the Vβ8.2 TCR is disrupted by coadministration with vectors expressing either IL-4 or -10. *J. Immunol.* 170: 765–774.
45. Kumar, V., and E. Sercarz. 1996. Genetic vaccination: the advantages of going naked. *Nat. Med.* 2: 857–859.
46. Cresswell, P., A. L. Ackerman, A. Giodini, D. R. Peaper, and P. A. Wearsch. 2005. Mechanisms of MHC class I-restricted antigen processing and cross-presentation. *Immunol. Rev.* 207: 145–157.
47. Houde, M., S. Bertholet, E. Gagnon, S. Brunet, G. Goyette, A. Laplante, M. F. Princiotta, P. Thibault, D. Sacks, and M. Desjardins. 2003. Phagosomes are competent organelles for antigen cross-presentation. *Nature* 425: 402–406.
48. Guermonprez, P., L. Saveanu, M. Kleijmeer, J. Davoust, E. P. Van Endert, and S. Amigorena. 2003. ER-phagosome fusion defines an MHC class I cross-presentation compartment in dendritic cells. *Nature* 425: 397–402.
49. Burgdorf, S., C. Scholz, A. Kautz, R. Tampe, and C. Kurts. 2008. Spatial and mechanistic separation of cross-presentation and endogenous antigen presentation. *Nat. Immunol.* 9: 558–566.
50. Touret, N., P. Paroutis, M. Terebiznik, R. E. Harrison, S. Trombetta, M. Pypaert, A. Chow, A. Jiang, J. Shaw, C. Yip, et al. 2005. Quantitative and dynamic assessment of the contribution of the ER to phagosome formation. *Cell* 123: 157–170.

51. Tompkins, S. M., J. R. Kraft, C. T. Dao, M. J. Soloski, and P. E. Jensen. 1998. Transporters associated with antigen processing (TAP)-independent presentation of soluble insulin to α/β T cells by the class Ib gene product, Qa-1(b). *J. Exp. Med.* 188: 961–971.
52. Bouwer, H. G., A. Bai, J. Forman, S. H. Gregory, E. J. Wing, R. A. Barry, and D. J. Hinrichs. 1998. *Listeria monocytogenes*-infected hepatocytes are targets of major histocompatibility complex class Ib-restricted antilisterial cytotoxic T lymphocytes. *Infect. Immun.* 66: 2814–2817.
53. Li, L., B. A. Sullivan, C. J. Aldrich, M. J. Soloski, J. Forman, A. G. Grandea III, P. E. Jensen, and K. L. Van. 2004. Differential requirement for tapasin in the presentation of leader- and insulin-derived peptide antigens to Qa-1b-restricted CTLs. *J. Immunol.* 173: 3707–3715.
54. Madakamutil, L. T., I. Maricic, E. Sercarz, and V. Kumar. 2003. Regulatory T cells control autoimmunity in vivo by inducing apoptotic depletion of activated pathogenic lymphocytes. *J. Immunol.* 170: 2985–2992.
55. Stanton, T. H., and S. Carbon. 1982. Gene(s) affecting the expression of Qa-1. *Immunogenetics* 16: 435–444.
56. Valitutti, S., S. Muller, M. Salio, and A. Lanzavecchia. 1997. Degradation of T cell receptor (TCR)-CD3-ζ complexes after antigenic stimulation. *J. Exp. Med.* 185: 1859–1864.
57. Yang,Y., R. B. Ratts, R. Z. Hussain, S. C. Northrop, L. H. Ben, A. Lovett-Racke, and M. K. Racke. 2007. CD28:B7 interaction is necessary for the protective effect of T cell vaccination in EAE. *Eur. J. Immunol.* 37: 2032–2042.
58. Liu, K., T. Iyoda, M. Saternus, Y. Kimura, K. Inaba, and R. M. Steinman. 2002. Immune tolerance after delivery of dying cells to dendritic cells in situ. *J. Exp. Med.* 196: 1091–1097.
59. Perruche, S., P. Zhang, Y. Liu, P. Saas, J. A. Bluestone, and W. Chen. 2008. CD3-specific antibody-induced immune tolerance involves transforming growth factor-β from phagocytes digesting apoptotic T cells. *Nat. Med.* 14: 528–535.
60. Kumar, V., R. Tabibiazar, H. M. Geysen, and E. Sercarz. 1995. Immunodominant framework region 3 peptide from TCR Vβ8.2 chain controls murine experimental autoimmune encephalomyelitis. *J. Immunol.* 154: 1941–1950.
61. Propato, A., G. Cutrona, V. Francavilla, M. Ulivi, E. Schiaffella, O. Landt, R. Dunbar, V. Cerundolo, M. Ferrarini, and V. Barnaba. 2001. Apoptotic cells overexpress vinculin and induce vinculin-specific cytotoxic T-cell cross-priming. *Nat. Med.* 7: 807–813.
62. Bevan, M. J. 2004. Helping the CD8$^+$ T-cell response. *Nat. Rev. Immunol.* 4: 595–602.
63. Murali-Krishna, K., J. D. Altman, M. Suresh, D. J. Sourdive, A. J. Zajac, J. D. Miller, J. Slansky, and R. Ahmed. 1998. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. *Immunity* 8: 177–187.
64. Schmied, M., H. Breitschopf, R. Gold, H. Zischler, G. Rothe, H. Wekerle, and H. Lassmann. 1993. Apoptosis of T lymphocytes in experimental autoimmune encephalomyelitis: evidence for programmed cell death as a mechanism to control inflammation in the brain. *Am. J. Pathol.* 143: 446–452.
65. Tabi, Z., P. A. McCombe, and M. P. Pender. 1995. Antigen-specific down-regulation of myelin basic protein-reactive T cells during spontaneous recovery from experimental autoimmune encephalomyelitis: further evidence of apoptotic deletion of autoreactive T cells in the central nervous system. *Int. Immunol.* 7: 967–973.
66. Walter, L., and M. L. Albert. 2007. Cutting edge: cross-presented intracranial antigen primes CD8$^+$ T cells. *J. Immunol.* 178: 6038–6042.
67. Calzascia, T., F. Masson, W. Di Berardino-Besson, E. Contassot, R. Wilmotte, M. Aurrand-Lions, C. Ruegg, P. Y. Dietrich, and P. R. Walker. 2005. Homing phenotypes of tumor-specific CD8 T cells are predetermined at the tumor site by crosspresenting APCs. *Immunity* 22: 175–184.
68. Kabelitz, D., T. Pohl, and K. Pechhold. 1993. Activation-induced cell death (apoptosis) of mature peripheral T lymphocytes. *Immunol. Today* 14: 338–339.
69. Bouillet, P., D. Metcalf, D. C. Huang, D. M. Tarlinton, T. W. Kay, F. Kontgen, J. M. Adams, and A. Strasser. 1999. Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. *Science* 286: 1735–1738.
70. Rawson, P. M., C. Molette, M. Videtta, L. Altieri, D. Franceschini, T. Donato, L. Finocchi, A. Propato, M. Paroli, F. Meloni, et al. 2007. Cross-presentation of caspase-cleaved apoptotic self antigens in HIV infection. *Nat. Med.* 13: 1431–1439.
71. Steinman, R. M., and J. Banchereau. 2007. Taking dendritic cells into medicine. *Nature* 449: 419–426.