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Plasmacytoid Dendritic Cells Enhance Mortality during Lethal Influenza Infections by Eliminating Virus-Specific CD8 T Cells

Ryan A. Langlois* and Kevin L. Legge*,

Previous studies have shown that the reduction in CD8 T cell immunity observed during high-dose influenza A virus (IAV) infection is mediated via lymph node (LN) dendritic cells (DCs) that express Fas ligand (FasL) and drive FasL-Fas (DC-T)–induced apoptosis. However, the specific DC subset(s) within the LN and the additional factors required for DC-mediated elimination of IAV-specific CD8 T cells remain unknown. In this paper, we demonstrate that plasmacytoid DCs (pDCs), which downregulate FasL, during sublethal, but not lethal, IAV infection, accumulate to greater numbers within the LNs of lethal dose-infected mice. Further our findings show that pDCs from lethal, but not sublethal, IAV infections drive elimination of Fas+ CD8 T cells and that this elimination occurs only in the absence of TCR recognition of IAV peptide-MHC class I complexes. Together, these results suggest that pDCs play a heretofore unknown deleterious role during lethal dose IAV infections by limiting the CD8 T cell response. The Journal of Immunology, 2010, 184: 4440–4446.

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direct viral-peptide MHC class I presentation. Taken together, these data suggest that pDCs are the cell type responsible for dampening the CD8 T cell response during lethal IAV infection in vivo and that such elimination occurs in the absence of cognate IAV Ag presentation.

Materials and Methods

Mice

Wild-type (WT) BALB/c mice were purchased from the National Cancer Institute (Frederick, MD). BALB/c CD90.1 congenic mice were kind gifts from Dr. Richard Elenow (Dartmouth College, Hanover, NH) and Dr. John T. Harty (University of Iowa, Iowa City, IA). Clone-4 (CL-4) TCR transgenic mice specific for the HA323/HA329 epitope of H1 and H2 IAV viruses, respectively, were a kind gift from Dr. Linda Sherman (The Scripps Research Institute, La Jolla, CA). BALB/c gld mice (CpT.C3-Tnfsf6gld/J) were kindly provided by Dr. Paul Allen (Washington University, St. Louis, MO). All experiments were performed in accordance with federal and institutional guidelines approved by the University of Iowa Animal Care and Use Committee.

Viruses

IAV-infected BALB/c mice 6–10 wk old were anesthetized by halothane or isofluorane and infected intranasally with either a 10 LD₅₀ or a 0.1 LD₅₀ dose of mouse-adapted A/PA-305/57 in 50 μl Iscoves media. Viruses were grown and stored as previously described (9).

MHC I tetramers

Tetramers HA308 (H-2Kd)LyQNVGTVYV, HA329 (H-2Kd)FYATVAGSL, and NP₃₅ (H2Kd)FYOVRALV were obtained from the National Institute of Allergy and Infectious Disease MHC Tetramer Core Facility (Atlanta, GA).

Flow cytometry

LN cells were stained with the following monoclonal Abs: rat anti-mouse CD8a (53-6.7), hamster anti-mouse CD11c (HL3), rat anti-mouse CD3ε (145-2C11), rat anti-mouse CD4 (CT-CD4), and rat anti-mouse CD43 (S7) purchased from BD Biosciences (San Jose, CA); mouse anti-mouse CD90.2 (5a-8), rat anti-mouse CD45R (RA3-6B2), rat anti-mouse DX5, and rat anti-mouse CD19 (6D6) purchased from CalTag (Invitrogen, Carlsbad, CA). Anti-FasL, CD95L (MFL3) was purchased from eBioscience (San Diego, CA). For FasL staining, cells were blocked with 1:100 rat serum, 1:100 hamster serum, and 1:400 free streptavidin (Molecular Probes, Eugene, OR) on ice for 25 min. Cells were then washed twice and stained with 2× biotin-conjugated anti-FasL (MFL3) followed by streptavidin-PE purchased from BD Biosciences. For surface staining, isolated cells (10⁶) were stained with Abs, and then fixed using BD FACs Lysing Solution (BD Biosciences). All conjugated Abs, and then fixed using BD FACS Lysing Solution (BD Biosciences). All experiments were performed using FlowJo software (TreeStar, Ashland, OR).

CD8 CL-4 T cell purification and adoptive transfer

Spleens from CL-4 mice were removed and processed into single-cell suspensions. Cells were labeled with anti-CD8a microbeads and purified according to the manufacturer’s instructions (Miltenyi Biotec, Auburn, CA). The purified CD90.2⁺CL-4 cells (2 × 10⁶) were then adoptively transferred i.v. into BALB/c CD90.1⁺ mice. At 24 h posttransfer, the host mice were infected intranasally with a 0.1 LD₅₀ IAV, as described above. For isolation of activated CL-4 CD8 T cells, lung draining LNs from IAV-infected CD90.1–CL-4 transferred host mice were removed on day 3 p.i. and digested, as described above. CD8 T cells were enriched using anti-CD8a beads according to the manufacturer’s instructions (Miltenyi Biotec). CD8a⁺ cells were then stained with Abs to CD90.2 and CD43, and activated CD90.2⁺CD43⁺ CL-4 cells were sort purified using a FACS DiVa (BD Biosciences).

LNDc purification

Lung draining LNs (peribronchior and mediastinal) were removed and digested with 4000 U type IV collagenase (Worthington Biochemical, Lakewood, NJ) and 600 U DNase I (Sigma-Aldrich, St Louis, MO) in Iscoves media for 10 min at room temperature. LNs were processed into a single-cell suspension and RBCs lysed using NH₄Cl-Tris. Cells were then stained with anti-CD3e–PE and anti-CD19–PE mAb, followed by anti-PE microbeads according to the manufacturer’s instructions (Miltenyi Biotec). Labeled cells were isolated using an autoMACS. The negative fraction was saved, stained with Abs to CD11c, CD45R (B220), CD8a, and CD4 and, with a FACs DiVa, sorted into CD11c⁺CD45R⁺CD8⁻ cells (i.e., pDCs); CD11c⁺CD45R⁻CD8⁻ (i.e., CD8a⁺ DCs); CD11c⁻CD45R⁻CD8⁻ (i.e., CD8a⁻ DCs); and CD11c⁻CD45R⁺CD8⁻ (i.e., DN DCs).

Ex vivo LNDc killing assay

Purified LNDc subsets (10⁶) were coincubated with 10⁶ activated CL-4 cells and 2.5 μg rmFas-human Fc (R&D Systems, Minneapolis, MN), 1 μM HA329 peptide (Bio-Synthesis, Lewisville, TX), or control media for 18 h at 37°C. To determine viability, cells were resuspended in 1× annexin binding buffer and stained with Annexin-V-APC and 7-aminocinomycin D (7-AAD) (40% per BD Biosciences’s recommendation for 10⁶ cells). The degree of DC killing of T cells was determined by measuring the fraction of CD8 T cells that were nonapoptotic (i.e., Annexin-V ⁰ 7-AAD ⁰), live cells and normalizing this value to CD8 T cells cultured alone.

In vivo pDC transfer studies

Spleens from naive WT or gld mice were removed, and single-cell suspensions stained with anti–PDCA-1 microbeads and purified according to the manufacturer’s instructions (Miltenyi Biotec). Then 2 × 10⁶ WT or gld pDCs were adoptively transferred i.v. into gld mice 18 h post lethal IAV infection. Some groups of pDCs were also pulsed with 1 μM HA329 peptide for 30 min at 37°C. Mice were monitored daily for weight loss and mortality, or on day 4 p.i. lung draining LNs were removed to determine IAV-specific T cell responses in the LNs.

FIGURE 1. pDCs and CD8a⁺ DCs modulate FasL expression during IAV infection. Mice were infected with either a 10 LD₅₀ (white portion of graph) or a 0.1 LD₅₀ (shaded portion of graph) of IAV, and on day 3 p.i. cells from draining LNs (pooled) from each group were examined for FasL expression. The dotted line represents staining with an isotype control mAb. A, FasL expression on CD11c⁺CD45R⁺CD8⁻ (i.e., pDCs); 10 LD₅₀ mean fluorescence intensity (MFI) = 60.5, and 0.1 LD₅₀ MFI = 28.3. B, FasL expression on CD11c⁺CD45R⁻CD8⁻ cells (i.e., CD8a⁺ DCs); 10 LD₅₀ MFI = 139, and 0.1 LD₅₀ MFI = 118. C, FasL expression on CD11c⁺CD45R⁻CD8⁻ cells (i.e., CD8a⁻ DCs); 10 LD₅₀ MFI = 618, and 0.1 LD₅₀ MFI = 496.2. The MFI of staining with isotype control mAb has been subtracted from the FasL MFI for the DC subsets, to yield the MFI reported above. Data are representative of five independent experiments.
Statistical analysis

Statistical analysis between two data sets was performed using a one-tailed Student t test. Differences were considered statistically significant at p values at or below 0.05. Statistical analysis for mortality experiments was performed using Kaplan–Meier survival analysis.

Results

CD8α+ DCs and pDCs downregulate FasL expression during lethal dose IAV infections

Our previous studies have demonstrated that reduction in IAV-specific CD8 T cell numbers in lethal dose IAV infections (Supplemental Fig. 1) is linked to FasL expression on DCs within the LNs (9). This FasL expression on LNDCs decreases during sublethal, compared with lethal, IAV infection, therein allowing activated effector IAV-specific CD8 T cells to leave the LNs and traffic to the lungs to fight the infection. However, the decrease in FasL expression on LNDCs during sublethal IAV infections was not uniform, with some LNDCs maintaining high levels of FasL expression. This finding suggested that a unique LNDC subset or subsets may be triggering the elimination of CD8 T cells during lethal dose IAV infection (9). To determine which LNDC subset or subsets differentially modulate FasL expression during IAV infections, mice were infected with either a lethal or a sublethal dose of IAV, and the level of FasL on individual LNDC subsets was determined on day 3 p.i., the time point where LNDC-mediated elimination of IAV-specific CD8 T cells begins (9). Interestingly, although both CD8α+ DCs and pDCs decreased FasL expression during sublethal compared with lethal dose IAV infection, CD8α+ DCs, including both the CD4+ and DN DC subsets, did not modulate FasL expression between the two IAV infection doses (Fig. 1). Further, the rDC subsets, which have migrated from the lungs to the LNs during IAV infection, remained FasL+ during both lethal and sublethal IAV infection (data not shown). Given that the pDC and CD8α+ DC subsets are the only LNDC populations to downmodulate FasL expression during sublethal dose IAV infections and that IAV-specific CD8 T cell responses are rescued at this dose of infection (Supplemental Fig. 1) despite the remaining CD8α+ DC FasL...
pDCs accumulate in the lung draining LNs in greater numbers during lethal dose IAV infections

Because our above results suggested the involvement of CD8α− DCs and/or pDCs in the induction of CD8 T cell apoptosis during lethal dose IAV infections, we next determined the kinetics and magnitude of their recruitment/expansion within the lung draining LNs during lethal and sublethal dose IAV infections. Our results show that the number of both pDC and CD8α+ DC increases within the LNs between days 2 and 4 (Fig. 2); that is, the time point during LNDC-mediated induction of CD8 T cell apoptosis occurs within the LNs (9). Specifically, the number of pDCs substantially increased in the LNs during lethal versus sublethal IAV infections between days 2 and 4, reaching significant differences by day 3 p.i. (Fig. 2A). Importantly, the number of pDCs present in the LNs was approximately six times greater than the number of CD8α+ DCs present during lethal dose IAV infections. These results, together with the fact that ~6500 activated CD43+CD8+ T cells were present in the LNs on day 4 p.i. during lethal IAV infection (data not shown), indicate that a putative in vivo E:T (i.e., DC/activated T cell) ratio for pDCs is conservatively ~1:1, compared with ~1:6 for CD8α+ DCs (data not shown). Given that pDCs exhibited enhanced LN recruitment during lethal dose IAV infections (Fig. 2) and that FasL expression on pDCs was dependent on the dose of IAV infection (Fig. 1), these data suggest that pDCs may be the predominant LNDC population responsible for elimination of IAV-specific CD8 T cells during lethal IAV infections.

dDCs directly kill IAV-specific CD8 T cells during lethal dose IAV infections

To directly determine the LNDC subset responsible for driving apoptosis of IAV-specific CD8 T cells during lethal dose IAV infections, we used an ex vivo apoptosis assay. Briefly, transgenic CD90.2+ clone-4 T cells (Cl-4), which are specific for the HA529 epitope of IAV, were transferred into CD90.1 hosts, which were then infected with a sublethal dose of IAV. Activated donor CD90.2+ CL-4 cells, which express higher levels of Fas (Supplemental Fig. 2), were then purified on day 3 p.i. (i.e., the time directly before T cell apoptosis occurs during lethal dose IAV infections) (9). These activated CD90.2+ CL-4 T cells were then incubated with CD90.1+ LNDC subsets purified from day 3 lethal dose IAV-infected mice. Subsequent CL-4 CD8 T cell apoptosis was measured following 18 h of coculture (Fig. 3). Interestingly, despite the fact that all lethal dose LNDC subsets express FasL at this point (Fig. 1), only pDCs induced statistically significant levels of apoptosis of the IAV-specific CL-4 CD8 T cells after coculture (Fig. 3B). The T cell apoptosis induced by pDCs was FasL-Fas dependent as coculture in
Given that pDCs during sublethal IAV infection downregulate FasL expression (Fig. 1), we next determined if pDCs from sublethal dose IAV-infected mice showed a similar ability to induce apoptosis of activated IAV-specific CD8 T cells. Consistent with their down-regulation of FasL during sublethal IAV infection, pDCs from sublethal dose IAV-infected mice were unable to induce apoptosis of IAV-specific CD8 T cells (Fig. 3D).

pDCs contribute to increased mortality during lethal IAV infection

Our previous studies have shown that gld mice (i.e., mice lacking functional FasL) exhibit increased IAV-specific CD8 T cell expansion and protection during lethal dose IAV infections (9). Therefore, we used gld mice as hosts for pDCs to directly determine if WT pDCs (i.e., Fasl+/+) could mediate a similar reduction in CD8 T cell responses in vivo. Indeed, when WT pDCs were adoptively transferred into gld mice, the number of CL-4 T cells within the LN on day 4 p.i. was significantly reduced (∼65%) (Fig. 4A). In fact, this reduction mirrored the number of IAV-specific T cells found in the LNs of lethal dose-infected WT mice. Further, when WT pDCs were transferred into lethal dose IAV-infected gld mice, it resulted in an enhanced mortality that was statistically similar to that in lethal dose IAV-infected WT mice (Fig. 4B). This increase in disease severity was Fasl dependent, as adoptive transfer of gld pDCs into lethal dose IAV-infected gld mice did not increase the severity of disease. Importantly, in these latter experiments, the transferred donor pDCs are the only cells expressing FasL and therefore the only LNDC population able to induce FasL–Fas–mediated apoptosis of IAV-specific CD8 T cells. Together, these data suggest that pDCs are sufficient to dampen in vivo the magnitude of the IAV-specific CD8 T cell response, ultimately leading to an enhanced mortality.

pDCs eliminate IAV-specific CD8 T cells through FasL–Fas interactions in the absence of cognate IAV Ag presentation

Given that both CD8α+ DCs and pDCs downregulate FasL expression in sublethal dose IAV-infected mice, it was surprising that only the pDCs eliminated IAV-specific CD8 T cells directly ex vivo. Further, both CD4 and DN DCs expressed FasL during lethal dose IAV infections yet did not lead to any detectable apoptosis. We therefore next undertook experiments to determine the mechanism regulating this differential killing. Previous studies have demonstrated that pDCs isolated directly ex vivo from IAV-infected mice are unable to stimulate naive CD8 T cells, suggesting that pDCs do not present IAV Ags via MHC class I in vivo (4, 5, 7, 15). In contrast to pDCs, CD8α+ DCs and CD8α− DCs purified from the LNs of IAV-infected mice are able to induce proliferation of naive CD8 T cells directly ex vivo (4, 7, 15). Given this differential ability of LNDC subsets to present IAV Ag to CD8 T cells, we hypothesized that IAV peptide–MHC I presentation might rescue the IAV-specific CD8 T cells from Fasl-mediated apoptosis. To test this hypothesis, activated CL-4 T cells were incubated with pDCs from the LNs of day 3 lethal dose IAV-infected mice in the presence or absence of exogenous IAV peptide, and the ability of pDCs to drive apoptosis of IAV-specific CD8 T cells was determined. Strikingly, culturing pDCs with IAV peptide abrogated their ability to eliminate IAV-specific CD8 T cells (Fig. 5A). To determine if Ag presentation by pDCs ablates their ability to eliminate IAV-specific CD8 T cells in vivo, pDCs were pulsed with IAV peptide and transferred into gld mice; the number of transferred CL-4 cells was then measured following IAV infection on day 4 p.i. Unlike unpulsed pDCs (Figs. 4A, 5B), pDCs pulsed with a cognate IAV peptide epitope did not eliminate IAV-specific CD8 T cells, leading to a similar T cell response to that observed in gld mice that did not receive pDCs (Fig. 5B). These data suggest that concomitant viral Ag peptide–MHC I presentation overrides the ability of pDCs to induce FasL–mediated apoptosis in vitro and in vivo and suggest that pDC elimination of IAV-specific CD8 T cells is critically tied to a lack of IAV Ag presentation. Therefore, the lack of apoptosis induction by CD8α+ and CD8α− DCs may relate to their presentation of IAV Ags. Consistent with this idea, pDCs from lethal dose IAV-infected mice were also able to mediate the apoptosis of in vitro activated DUC18 (i.e., a non–IAV-specific T cell that displays an activation phenotype similar to that of in vivo activated CL-4 T cells; Fig. 6B, Supplemental Fig. 3), similar to their elimination of in vitro activated CL-4 T cells (Fig. 6A). Furthermore, although CD8α− DCs purified from IAV-infected mice were unable to eliminate IAV-specific transgenic T cells (Figs. 3, 6A), they were capable of eliminating non–IAV-specific transgenic T cells (Fig. 6B), suggesting that CD8α− DCs can mediate elimination of activated T cells in the absence of Ag presentation. Together these data support the idea that elimination of IAV-specific T cells during lethal IAV infection does not require engagement of TCRs and in fact TCR engagement may inhibit such apoptosis. Therefore, CD8 T cell apoptosis may instead relate to the overall T cell activation state and Fas expression.

Discussion

DC elimination of T cells through FasL–Fas interactions has been previously described by multiple investigators (19–21). Süss and Shortman (20) demonstrated that CD8α− splenic DCs expressing FasL were able to eliminate CD4 T cells during an MLR. In addition, multiple groups have used adoptive transfer of DCs transfected with FasL to control T cell numbers in a variety of disease settings, including autoimmunity, cancer, and viral infection (19, 21, 22). In this paper, we have shown that LN resident pDCs can mediate the elimination of IAV-specific CD8 T cells during lethal dose IAV infections. Together, our results suggest that elimination of activated Fas+ T cells by Fasl+ pDCs may represent an integral mechanism for dampening T cell numbers.

FIGURE 6. pDCs from lethal dose IAV-infected mice eliminate IAV-specific and non–IAV-specific activated CD8 T cells. CD8 T cells from the spleens of naive CL-4 (A) and DUC18 (B) transgenic mice were purified and cultured for 3 d in the presence of αCD3/CD28 Abs to activate the T cells. Activated transgenic T cells (105) were incubated with pDCs or CD8α− DCs (105) from lethal dose IAV-infected mice at 37°C for 18 h, as described in Fig. 3. After incubation, the percentage of CD90.2+Annexin V−7-AAD− live transgenic T cells was determined and normalized to CD8 T cells incubated alone. Data are representative of two independent experiments. n.s. = p > 0.1.
Whereas DC-mediated reduction of the effector T cell response in autoimmune reactions or at the conclusion of an immune response would be beneficial, the loss of effector CD8 T cells is clearly detrimental to survival during a high-dose IAV infection (9). Indeed, our previous studies have shown that in the absence of functional FasL, sufficient numbers of CD8 T cells develop to control the high-dose IAV inoculum (Fig. 4) (9). Thus, enhanced recruitment of pDCs into the LNs observed during lethal dose IAV infections (Fig. 2), coupled with pDC-mediated elimination of the IAV-specific CD8 T cell response (Figs. 3, 4), allows the virus to escape adaptive immune control, leading to death of the infected host. In this regard, recent studies have shown that individuals infected with highly pathogenic avian (H5N1) IAV have dampened or reduced CD4 and CD8 T cell responses (23). Similarly, mice and monkeys infected with highly pathogenic H5N1 IAV develop T cell lymphopenia (24–26), with loss of CD8 T cells in the lungs and lymph nodes, associated with enhanced levels of apoptosis (24). The exact pathway or pathways and cell type mediating the apoptosis responsible for T cell lymphopenia remain poorly understood at this time. However, given our results, it will be important to determine what role FasL expression by pDCs plays in the lymphopenia associated with highly pathogenic avian H5N1 IAV infections.

Surprisingly, LNDC elimination of IAV-specific CD8 T cells does not require cognate MHC class I Ag presentation. In fact, the rescue of these T cells from FasL+ DC-induced apoptosis during recognition of viral peptide-MHC I complexes may be due in part to TCR-mediated upregulation of NK-xB, which also has been shown to protect T cells, macrophages, and B cells from Fas-mediated apoptosis (27–30). Interestingly, signals through the BCR, which upregulate NF-xB, result in transient (∼24 h) protection from Fas-mediated apoptosis (28). In our studies, pDC-driven apoptosis occurs in the LNs at a time point concomitant with DC-mediated Ag presentation. Signaling through Fas on T cells during activation can act as a costimulatory molecule and has been demonstrated to increase T cell proliferation and activation (31–34). Together these findings suggest the possibility that during a narrow window immediately following the activation of naive T cells within the LNs, cognate MHC class I Ag–TCR interactions in the presence of FasL–Fas engagement may lead to enhanced T cell responses rather than apoptosis. Conversely, FasL–Fas engagement alone or after egress from the draining LNs would mediate death. Consistent with our results showing that coculturing of pDCs and CL-4 T cells with IAV peptide epitopes reverses the pDC-mediated loss of T cells (Fig. 5A), a recent report has demonstrated that Ag-pulsed FasL-transfected DCs enhance Ag-specific CD8 T cell responses, rather than induce apoptosis. This finding suggests that the presentation of Ag by these transfected DCs inhibits their ability to drive elimination of cognate T cells (35). In contrast to this report, and our own results, other groups have suggested that DC FasL-mediated elimination of CD4 and CD8 T cells can occur in an Ag-dependent manner (19, 21, 36). The reason for these differences is not clear at this time; however, in contrast to our own studies, these latter experiments used conventional bone marrow-derived DCs or DC cell lines, rather than pDCs obtained from the LNs. In addition, these studies used effector DCs transfected with FasL cDNA, resulting in constitutively high levels of FasL.

Our results suggest that pDC-mediated induction of apoptosis in activated T cells during lethal dose IAV infections occurs independently of IAV Ag presentation (Fig. 5). In agreement with this idea, recent reports have demonstrated that although LN resident pDCs contain IAV proteins following infection, they are unable to stimulate naive or memory CD8 T cells and may instead regulate B cell responses (5). Unlike pDCs, LN resident CD8α+ DCs acquire IAV Ag (likely from migratory rDCs) and cross-present this Ag during IAV infections (15). Interestingly, our results show that although CD8α+ DCs are also able to regulate FasL expression in an IAV dose-dependent manner (Fig. 1), they do not eliminate IAV-specific CD8 T cells during lethal dose IAV infections (Fig. 3). This characteristic appears to be due to their presentation of viral peptides, as our preliminary results suggest that CD8α+ DCs from lethally infected β2m−/− mice induce substantial apoptosis of IAV-specific T cells (data not shown). Together, these results, along with those demonstrating that pDCs from lethal dose IAV-infected mice kill both activated non–IAV-specific and IAV-specific CD8 T cells with the same efficiency (Fig. 6A), indicate that pDC elimination of effector CD8 T cells during lethal dose IAV infections is independent of TCR engagement. Rather, pDC-mediated elimination of T cells is associated with the T cell activation state and Fas expression. Thus, Fas-expressing activated or memory CD8 T cells of any specificity might be susceptible to pDC-mediated apoptosis during lethal dose IAV infections.

In addition to differential FasL expression on pDCs from lethal and sublethal dose IAV-infected mice, our results demonstrate an enhanced recruitment of pDCs into the LNs of lethal dose-infected mice (Fig. 2). pDC recruitment into LNs through high endothelial venules and enter the LNs, the increased recruitment of pDCs during lethal IA V infection—namely, the elimination of activated CD8 T cells during IAV infections (15). Interestingly, our results show that although CD8α+ DCs are also able to regulate FasL expression in an IAV dose-dependent manner (Fig. 1), they do not eliminate IAV-specific CD8 T cells during lethal dose IAV infections (Fig. 3). This characteristic appears to be due to their presentation of viral peptides, as our preliminary results suggest that CD8α+ DCs from lethally infected β2m−/− mice induce substantial apoptosis of IAV-specific T cells (data not shown). Together, these results, along with those demonstrating that pDCs from lethal dose IAV-infected mice kill both activated non–IAV-specific and IAV-specific CD8 T cells with the same efficiency (Fig. 6A), indicate that pDC elimination of effector CD8 T cells during lethal dose IAV infections is independent of TCR engagement. Rather, pDC-mediated elimination of T cells is associated with the T cell activation state and Fas expression. Thus, Fas-expressing activated or memory CD8 T cells of any specificity might be susceptible to pDC-mediated apoptosis during lethal dose IAV infections.

The data presented in this paper describe a novel role for pDCs during lethal dose IAV infection—namely, the elimination of activated CD8 T cells, leading to enhanced mortality. Given the emerging threat of highly pathogenic pandemic IAV and the detrimental role FasL+ pDCs can play during a lethal IAV infection, our findings suggest that pDCs and FasL may be strong candidates for therapeutic blockade during highly virulent IAV infections.

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Disclosures
The authors have no financial conflicts of interest.

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