Dendritic Cells Undergo Rapid Apoptosis In Vitro During Antigen-Specific Interaction with CD4+ T Cells

Hiroyuki Matsue, Dale Edelbaum, Aubrey C. Hartmann, Akimichi Morita, Paul R. Bergstresser, Hideo Yagita, Ko Okumura and Akira Takashima

*J Immunol* 1999; 162:5287-5298; 
http://www.jimmunol.org/content/162/9/5287
Dendritic Cells Undergo Rapid Apoptosis In Vitro During Antigen-Specific Interaction with CD4\(^+\) T Cells

Hiroyuki Matsue, Dale Edelbaum,* Aubrey C. Hartmann,* Akimichi Morita,* Paul R. Bergstresser,* Hideo Yagita,† Ko Okumura,† and Akira Takashima*‡

The terminal fate of dendritic cells (DC) remains relatively uncertain. In this study, we tested the hypothesis that DC undergo apoptosis after Ag-specific interaction with T cells. When splenic DC isolated from BALB/c mice were cocultured with HDK-1 T cells (a keyhole limpet hemocyanin (KLH)-specific CD4\(^+\) Th1 clone) in the presence of KLH, they showed conspicuous cell death as measured by propidium iodide (PI) uptake and chromatin condensation, whereas they remained relatively intact when incubated with either T cells or KLH alone. Likewise, the long term DC line XS52, which was established from BALB/c mouse epidermis, also died rapidly (within 2 h), and they exhibited characteristic DNA laddering when cocultured with HDK-1 T cells in the presence of KLH. RT-PCR and FACS analyses revealed the expression of CD95 (Fas) by XS52 DC and of CD95 ligand (CD95L) by activated HDK-1 T cells, suggesting a functional role for these molecules. In fact, anti-CD95L mAb inhibited partially (50%) T cell-mediated XS52 cell death, and coupling of surface CD95 with anti-CD95 mAb triggered significant XS52 cell death, but only in the presence of cycloheximide. Thus, ligation of CD95 (on DC) with CD95L (on T cells) is one, but not the only, mechanism by which T cells induce DC death. Finally, DC isolated from the CD95-deficient mice were found to be significantly more efficient than DC from control mice in their capacity to induce delayed type hypersensitivity responses in vivo. We propose that T cell-induced DC apoptosis serves as a unique down-regulatory mechanism that prevents the interminable activation of T cells by Ag-bearing DC. The Journal of Immunology, 1999, 162: 5287–5298.

Steinman and coworkers have established the concept that immunologically naive T cells can be activated most effectively by a special subset of Ag presenting leukocytes, termed dendritic cells (DC)\(^4\) (1, 2). DC have been shown to play essential roles in the induction of T cell-mediated immune reactions against a wide variety of Ag, including chemical haptens, foreign proteins, infectious pathogens, and tumor-associated Ag (1, 3–5). Recent studies have revealed several activities or properties that allow DC to perform this task efficiently: 1) DC incorporate exogenous Ag effectively by pinocytosis and phagocytosis (6) using cell surface receptors (e.g., macrophage mannose receptor, DEC-205, Fc receptors) (7); 2) on internalization, Ag are delivered into special cytoplasmic compartments (called MHC class II-rich vesicles or MHC class II compartment), where Ag are processed into antigenic peptides and coupled to MHC class II molecules (7); 3) DC are unique in their ability to migrate from peripheral locations to T cell areas of lymph nodes (8, 9); 4) DC express relatively high levels of MHC class II molecules and important costimulatory molecules, including CD80, CD86, and CD40, equipping them to deliver T cell activation signals (10–12); 5) DC produce several proinflammatory and T cell-stimulatory cytokines, i.e., IL-1\(\beta\), IL-6, and IL-12 (13–17), and they secrete several chemokines, i.e., IL-8, macrophage-inflammatory protein (MIP)-1\(\alpha\) and MIP-1\(\gamma\), and DC-CK, which attract T cells (18–21). In sum, DC possess all phenotypic and functional properties required for the efficient induction of cellular immunity.

DC do not simultaneously exhibit all the properties described above; rather they acquire them sequentially, apparently when needed. For example, DC-lineage Langerhans cells (LC) in skin are relatively deficient in their capacity to activate naive T cells when tested immediately after isolation from skin, but they acquire this capacity during short term culture in vitro. This capacity is supported by an elevated expression of MHC class II and costimulatory molecules and by increased production of several cytokines (22). At the same time, cultured LC lose other properties, including pinocytosis, phagocytosis, and the ability to process complex protein Ag (1, 6, 9). These changes in LC function are thought to reflect their “maturation” from DC specialized for Ag uptake and processing into DC specialized for delivering T cell-stimulatory signals (9, 22). Importantly, LC undergo a similar maturation process in vivo following topical application of reactive haptens (23–25), the treatment that also triggers LC migration into draining lymph nodes (26). Therefore, when LC reach lymph nodes, they most likely have completed their transition into the T cell-stimulatory DC. This transition illustrates the dynamic nature of DC biology; DC change both phenotype and function in a life cycle, depending on their maturation states and location.

The ultimate question concerns the terminal fate of DC after Ag presentation has been accomplished. One scenario is that they reside in lymph nodes for relatively long periods of time, ensuring...
maximal activation of T cells. Perhaps they leave lymph nodes and return to their tissues of origin. Even more remote is the possibility that DC are killed by the very T cells that they activate. Ingulli et al. (27) developed a unique experimental system to follow the migration and the fate of DC. In this system, splenic DC were pulsed with OVA, labeled with a fluorescent dye, and then injected s.c. into syngeneic mice that had received an adoptive transfer of OVA-reactive, naive CD4+ T cells from transgenic animals. Ingulli et al. observed that the DC migrated into draining lymph nodes where they interacted closely with CD4+ T cells. Interestingly, these infused DC disappeared rapidly (by 48 h) from the lymph nodes at the time when clonal expansion of OVA-reactive T cells became detectable. However, these in vivo experiments failed to determine whether those DC had died or whether they had migrated out of the lymph nodes. In the present study, we developed in vitro experimental systems to determine the terminal fate of DC. Our results document that DC undergo rapid apoptosis upon Ag-specific interaction with a CD4+ T cell clone.

Materials and Methods

Animals and cells

BALB/c mice, B6.MRL-FAStm mice, and C57BL/6J mice (all 6–8 wk old, female) were purchased from The Jackson Laboratory (Bar Harbor, ME). Splenic DC were isolated as described previously (28). Briefly, spleen cell suspensions were prepared by mechanical dissociation using fine forceps, followed by collagenase treatment (1% collagenase, 1 h, 37°C). After lysis of erythrocytes, splenic cells were subjected to gradient centrifugation with Percoll (Pharmacia, Piscataway, NJ); cells collected from the interface between 1.035 and 1.075 g/ml of Percoll were then incubated on tissue culture plates. After a 90-min incubation, nonadherent cells were removed by extensive pipetting, and the adherent cells were cultured overnight. Cells released during the second culture period were harvested and used as splenic DC preparations; they contained 40–50% CD11c+ DC, 30–40% B220+ B cells, 5–10% CD3+ T cells, and <5% of pan-NK mAb DX-5+ NK cells assessed by FACS analysis. XS52 is a long term DC line we established from the epime-ris of a newborn BALB/c mouse (29). XS52 cells were maintained and expanded in complete RPMI in the presence of GM-CSF (2 ng/ml) and NS47 fibroblast supernatant (10%). Phenotypic and functional features of this DC line are described elsewhere (29–33). As responder T cells, we used the HDK-1 cells, which are a keyhole limpet hemocyanin (KLH)-specific CD45+ T cells, and they exhibited significant proliferative responses to KLH-pulsed spleen cells and expanded by repeated feeding with IL-2 (200 U/ml). In some experiments, we used a short term cultured KLH-reactive CD4+ T cell line. Briefly, BALB/c mice were immunized by s.c. injection of 100 μg of KLH in PBS emulsified in CFA into the foot pads. After 7 days, T cells were isolated from the draining lymph nodes, and cultured in the presence of irradiated syngeneic spleen plus KLH (100 μg/ml) and IL-2 (25 U/ml). The resulting populations were >95% CD4+, and they exhibited significant proliferative responses to KLH-pulsed XS52 DC.

Apoptosis assays

XS52 DC (1 × 106 cells/ml) or splenic DC (1 × 106 cells/ml) were cocultured with HDK-1 T cells (4 × 104 or 1 × 104 cells/ml, respectively) in the presence of KLH (100 μg/ml). Unless otherwise mentioned, all incubations were conducted in 5-ml tissue culture tubes using complete RPMI in the absence of added growth factors. In some experiments, XS52 DC or HDK-1 T cells were prelabeled with FITC (100 μg/ml, 10 min, 4°C) before coculture (33). Alternatively, XS52 DC and HDK-1 T cells were first incubated together and then postlabeled with FITC-conjugated mAb against Iaα, Thy-1.2, or CD11c (PharMingen, San Diego, CA). At various intervals during coculture, cells were collected by centrifugation, labeled with propidium iodide (20 μg/ml) and analyzed by FACS (Beckton Dickinson, Mountain View, CA) (35). To identify apoptotic DC visually, samples were postlabeled with PE-conjugated anti-Iaα mAb (PharMingen), fixed with 3% paraformaldehyde, and then stained with 10 μg/ml Hoechst 33342 (Sigma, St. Louis, MO). We then counted the numbers of Iaα+ DC showing the condensed nuclei under fluorescence microscopy at ×400. To examine the role of CD95L, XS52 DC were cocultured with HDK-1 T cells and KLH in the continuous presence of 10 μg/ml anti-CD95L mAb (MFL-1) (36), anti-CD48 mAb (PharMingen), or control hamster IgG (PharMingen). To examine the role of CD54, we added anti-CD54 mAb (PharMingen) or rat IgG2a control (PharMingen). DNA degradation was examined as described previously with slight modification (35, 37). Briefly, XS52 cells were labeled with [3H]thymidine (5 μCi/ml) for 48 h, washed extensively, and then cocultured for 8 h in the presence or absence of HDK-1 T cells and/or KLH. Subsequently, these cells were collected by centrifugation and then lysed in a buffer consisting of 10 mM Tris-HCl, 10 mM EDTA and 0.2% Triton X-100 (pH 7.5). The lysate was centrifuged (13,000 × g) for 10 min at 4°C, and low m.w. DNA was then purified from the supernatant by phenol-chloroform extraction followed by ethanol precipitation. DNA was separated in a 2% agarose gel and then transferred onto a nitro cellulose membrane (Bio-Rad, Hercules, CA). The membrane was exposed to Kodak x-ray film (Eastman Kodak, Rochester, NY) at −80°C for 10 days.

Assays for CD95 and CD95 ligand (CD95L) expression

XS52 DC and HDK-1 T cells were labeled with anti-CD95 mAb (Jo2, PharMingen) or anti-CD95L mAb (MFL-1) and then incubated with PE-conjugated goat anti-hamster IgG (Jackson ImmunoResearch, West Grove, PE). In some experiments, the cells were stimulated with LPS (100 ng/ml) or Con A (4 μg/ml) for 8 h before analysis. To examine CD95 and CD95L mRNA expression, total RNA isolated from these cells were subjected to RT-PCR using the following primers: 5′-ATGCAATCTCGGGCTK-3′ and 5′-CTCAGGCGTCATCTGTCC-3′ for CD95, and 5′-AGCTATCCTGGGGGCGATT-3′ and 5′-ATGACCGACATTAG GACCAC-3′ for CD95L. Primers for IL-1β, IFN-γ, and β-actin were purchased from Clontech Laboratories (Palo Alto, CA). PCR products were harvested within the linear range of amplification (30 cycles for CD95, CD95L, IFN-γ, and IL-1β, and 25 cycles for β-actin) and then separated on 1% agarose gel containing ethidium bromide. In some experiments, PCR products were analyzed further by Southern blotting using the internal oligo probes (5′-AAAACAAATGCGACCTTGACC-3′ for CD95 and 5′-TCTAGGCGACCAGGAATATT-3′ for CD95L), as described previously (38).

Induction of DTH response

Splenic DC isolated from B6.MRL-FAStm mice and from control C57BL/6J mice were pulsed with KLH (100 μg/ml) during the overnight incubation period. After extensive washing, the KLH-pulsed DC were suspended in PBS and injected s.c. into C57BL/6J mice. As a positive control, a different group of mice were immunized by s.c. injection of KLH plus CFA. Ten days later, these animals were challenged by s.c. injection of KLH (50 μg/20 μl PBS/mouse) into the left hind footpad. The same volume of saline alone was injected into the right hind footpad as control. Footpad swelling responses were measured at 24 and 48 h using a calliper type engineer’s micrometer (Mitsutoyo, Kawasaki, Japan).

Results

Splenic DC death during Ag-specific interaction with CD4+ T cells

In the first set of experiments, we examined the fate of splenic DC during Ag-specific interaction with CD4+ T cells. We isolated DC from BALB/c mouse spleens with a standard protocol that used centrifugation through Percoll gradient, removal of plastic nonadherent cells, and overnight culture (28). As a responder T cell population, we used a KLH-specific, CD4+ Th1 clone HDK-1. Splenic DC preparations were cocultured with HDK-1 T cells for an additional 20 h in the presence or absence of Ag (KLH), labeled with FITC-conjugated anti-CD11c mAb, and then examined for viability by PI uptake. As noted in Fig. 1A, ~50% of the CD11c+ cells were judged to be PI positive after the second (20 h) incubation even in the absence of either HDK-1 cells or KLH. Cell viability as measured by trypan blue exclusion remained relatively high during the isolation procedure, i.e., >95% in low density spleen cells after gradient centrifugation and 80–85% in DC preparations after the first overnight culture. On the other hand, 35–40% of CD11c+ cells in the same DC preparations were judged to be “dead” by the more sensitive FACS-based assay of PI uptake. We interpreted these observations to indicate that CD11c+ splenic...
DC undergo spontaneous cell death during prolonged culture periods, with 35–40% death during the isolation procedure and additional 10–15% death during the second 20-h incubation. Importantly, the frequency of PI-positive CD11c⁺ splenic DC increased to 90% after coincubation with both HDK-1 T cells and KLH, whereas incubation with either T cells or KLH alone had only modest effects (Fig. 1A). In six independent experiments, relatively consistent numbers of CD11c⁺ splenic DC (85–91%, 87.3 ± 2.9%) were judged to be PI positive after 20 h of incubation with T cells and KLH; these values were statistically higher (p < 0.01, Student’s t test) than those observed in any of the control groups, DC, T cells, DC + KLH, or DC alone (Fig. 1B). These observations indicate that splenic DC undergo spontaneous apoptosis in culture and that Ag-specific interaction with CD4⁺ T cells augments this cell death.

Characterization of T cell-mediated DC death

To study the process of DC apoptosis in detail and to identify its mechanism(s), we developed a better defined experimental system using the long term DC line XS52. We observed previously that XS52 DC are at least as potent as splenic DC in their capacity to present KLH to the HDK-1 T cells (29). As shown in Fig. 3A, XS52 cells showed only modest (~10%) spontaneous cell death (assessed by PI uptake) after 16 h of incubation in the absence of added growth factors. Likewise, the majority of HDK-1 T cells remained viable over the same time period. When XS52 DC and HDK-1 T cells were incubated together in the presence of KLH (i.e., “complete” coculture), substantial numbers of these cells became PI positive. By contrast, they remained mostly viable when incubated together in the absence of KLH or incubated individually in the presence of KLH (i.e., “incomplete” coculture), substantial numbers of these cells became PI positive. These results suggest strongly that splenic DC undergo spontaneous apoptosis in culture and that this process is augmented significantly by Ag-specific interaction with CD4⁺ T cells.

FIGURE 1. Splenic (Sp) DC death during Ag-specific interaction with CD4⁺ T cells. Splenic DC (1 × 10⁶ cells/ml) isolated from BALB/c mice using a standard, overnight culture protocol were cocultured for an additional 20 h with HDK-1 T cells (1 × 10⁶ cells/ml) in the presence or absence of 100 μg/ml KLH. The samples were labeled with FITC-conjugated anti-CD11c mAb and then examined for PI uptake. Data in A are representative histograms of PI uptake (FL-2 channel) by the CD11c⁺ populations and the percentages of PI-positive cells (numbers above the histograms). Data in B are means ± SD from six independent experiments. The percentage of cell death in CD11c⁺ populations was significantly (p < 0.01) higher in the cultures that included both T cells and KLH than in any other (control) cultures.
Mechanisms of T cell-mediated DC apoptosis

The observations of chromatin condensation among splenic DC and the rapid onset of XS52 DC death both supported the hypothesis that DC die by an apoptotic mechanism. To test this hypothesis more directly, we examined DNA degradation in XS52 DC. XS52 cells were first labeled with [3H]thymidine, washed extensively, and then incubated for 8 h in the presence or absence of HDK-1 T cells and/or KLH. Low m.w. DNA isolated from complete cocultures showed characteristic DNA laddering in autoradiography, whereas DNA remained relatively intact when the XS52 cells were incubated by themselves or with either HDK-1 cells or KLH alone (Fig. 4). Taken as a whole, our observations indicate that DC do undergo apoptosis after Ag-specific interaction with CD4+ T cells.

During Ag-dependent interaction, not only do XS52 DC deliver activation signals to HDK-1 T cells but they also receive signals back from T cells. This bidirectional signaling triggers various changes in both populations, e.g., IFN-γ secretion by HDK-1 T cells and IL-1β secretion by XS52 cells (32). To determine whether such signaling was also required to induce XS52 cell death, we blocked Ag-dependent interaction with anti-Ia mAb. As shown in Fig. 5B, anti-Ia mAb inhibited the secretion of IFN-γ and IL-1β in the complete cocultures, corroborating our previous observations (32). Importantly, anti-Ia mAb also inhibited XS52 cell death significantly in the same complete coculture, whereas control IgG showed minimal effects (Fig. 5A and B). We interpret these observations to indicate that MHC class II-dependent DC-T cell interaction is required for delivering an apoptotic signal.

Ligation of CD95 with CD95L is now known to play a crucial role in triggering apoptosis in many experimental systems (42–51). Importantly, XS52 cells were observed to express constitutively both CD95 and CD95L mRNA, as detected by RT-PCR (Fig. 6A). Treatment with LPS, which activated XS52 cells as judged by increased IL-1β mRNA expression, failed to significantly modulate mRNA expression for either CD95 or CD95L. On the other hand, HDK-1 T cells expressed constitutively both CD95 and CD95L mRNA. Treatment with Con A, which activated XS52 cells as judged by up-regulated IL-1β mRNA expression, showed minimal effects on CD95L mRNA expression, but it induced up-regulation of CD95 mRNA expression (Fig. 6A). These observations were confirmed by Southern blot analysis of the PCR products (Fig. 6B). By FACS analyses, we observed that XS52 DC express CD95, but not CD95L, regardless of their activation states, whereas HDK-1 T cells express CD95L only after activation (Fig. 7). Thus, it appeared that surface expression of CD95 and CD95L is highly regulated by posttranscriptional mechanisms.
Our failure to detect surface expression of CD95 on activated HDK-1 T cells raised the question whether XS52 DC might respond differently when incubated with conventional CD4\(^+\) T cells that would express CD95 on activation. To address this question, we obtained a KLH-reactive, short term cultured CD4\(^+\) T cell line from the lymph node of BALB/c mice that had been immunized with KLH/CFA. As shown in Fig. 8A, those T cells expressed CD95 on their surface after Con A activation. When incubated with these T cells in the presence of KLH, XS52 DC underwent significant cell death. Once again, only modest cell death was observed with XS52 DC after incubation with T cells alone or KLH alone, duplicating our observations with the HDK-1 T cell clone (Fig. 8B).

Our detection of CD95 on XS52 DC and CD95L on activated HDK-1 T cells led us to test the functional role of CD95/CD95L system in T cell-mediated DC apoptosis. As shown in Fig. 9, anti-CD95 mAb Jo2 induced significant death of thymocytes within 24 h, and thymocyte death was further augmented by

Table I. XS52 DC death during Ag-specific interaction with CD4\(^+\) T cells

<table>
<thead>
<tr>
<th>Labeling Cells</th>
<th>% Cell Death (no KLH)</th>
<th>% Cell Death (with KLH)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>XS52</td>
<td>HDK-1</td>
</tr>
<tr>
<td>Prelabeling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FITC</td>
<td>7.6 ± 3.1</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td>Anti-Ia</td>
<td>16.8 ± 3.7</td>
<td>7.1 ± 1.5</td>
</tr>
<tr>
<td>Anti-Thy-1</td>
<td>15.9 ± 7.6</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>Postlabeling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FITC</td>
<td>12.8 ± 6.8</td>
<td>5.8 ± 4.8</td>
</tr>
</tbody>
</table>

* In prelabeling protocols, either XS52 DC or HDK-1 T cells were prelabeled with FITC and then cocultured with the other cell type (HDK-1 T cells or XS52 DC, respectively) in the presence or absence of KLH. After 16 h of coculture, samples were examined for PI uptake by FACS. In postlabeling protocols, XS52 and HDK-1 T cells were cocultured first in the presence or absence of KLH, labeled with either FITC-anti-Ia mAb or FITC-anti-Thy-1 mAb, and examined for PI uptake. Data are the means ± SD of percentage of cell death from three independent experiments.
cycloheximide (CHX), an inhibitor of protein synthesis, corroborating the reports of others (52, 53). The same anti-CD95 mAb induced significant XS52 cell death only in the presence of CHX. This is also consistent with previous reports that CHX augments, or is even required, for anti-CD95-triggered apoptosis (52, 54, 55). These results suggest that thymocytes and DC undergo apoptosis by CD95-dependent, but perhaps different, mechanisms. To determine the extent to which T cell-dependent DC apoptosis depends on the CD95/CD95L pathway, we added neutralizing mAb against CD95L in complete cocultures. As shown in Fig. 10, this mAb inhibited significantly, albeit partially (~50%), HDK-1 T cell-induced XS52 cell death. The extent of inhibition remained at ~50%, even with higher concentrations (up to 30 μg/ml) of anti-CD95L (data not shown). The specificity of inhibition with anti-CD95L mAb was verified by the observations that neither a conventional hamster IgG control nor anti-CD48 mAb (hamster IgG) that bound to the XS52 cell surface (data not shown) blocked XS52 cell death significantly (Fig. 10). We also tested whether the same anti-CD95L mAb would inhibit T cell-induced apoptosis of splenic DC. As shown in Fig. 11, anti-CD95L mAb, but not anti-CD48 mAb, prevented apoptosis of CD11c+ splenic DC triggered by Ag-specific interaction with HDK-1 T cells. Thus, we conclude that ligation of CD95 on DC with CD95L on activated T cells is one of the mechanisms leading to DC apoptosis.

**FIGURE 4.** XS52 DC exhibit DNA fragmentation after Ag-specific interaction with T cells. XS52 cells (2 × 10^6 cells per group) were first labeled with [³H]thymidine for 48 h, washed extensively, and then incubated for an additional 8 h in the presence or absence of HDK-1 T cells (8 × 10^5 cells) and/or KLH (100 μg/ml). Low m.w. DNA isolated from each sample was examined for DNA profiles. Data are representative autoradiographic DNA profiles from three independent experiments.

**FIGURE 5.** Inhibition of T cell-induced XS52 DC death by anti-Ia mAb. XS52 cells (2 × 10^5 cells/ml) were incubated for 8 h in the presence or absence of FITC-labeled HDK-1 T cells (8 × 10^5 cells/ml) and/or KLH (100 μg/ml). Anti-Ia mAb (MK-D6, 10 μg/ml) or control mouse IgG2a (10 μg/ml) were added in these cultures. Data in A are representative histograms of PI uptake (FL-2 channel) within the FITC-negative population (i.e., XS52 cells). Data in B are means ± SD (n = 3) of percentage of PI-positive cells within the FITC-negative populations and the amounts of IFN-γ and IL-1β detected in the supernatants of these cultures. Anti-Ia mAb caused significant (p < 0.01) inhibition of each parameter as compared with control IgG.
As shown in Fig. 12A, CD54 and its ligand LFA-1 (CD11a/CD18) are both expressed by XS52 DC and by HDK-1 T cells, suggesting that these adhesion molecules may play a functional role in T cell-induced DC apoptosis. In fact, anti-CD54 mAb inhibited XS52 DC apoptosis in a dose-dependent manner, with 40% inhibition achieved at 10 μg/ml. An isotype-matched control IgG had minimal effects even at the highest concentration tested. Not only do these observations indicate the involvement of CD54-CD11a/CD18 interaction in T cell-induced DC apoptosis, they also suggest that one may be able to manipulate DC apoptosis experimentally using anti-CD54, anti-CD95L, and/or other mAb.

In vivo relevance of CD95/CD95L-mediated, T cell-induced DC apoptosis

Our observations that DC undergo apoptosis upon Ag-specific interaction with T cells imply that this may be a mechanism that limits the extent of DC-induced T cell activation in vivo. To test this hypothesis, we used CD95-deficient animals (B6.MRL-Fas" mice). Splenic DC isolated from the CD95-deficient mice and from wild-type mice (C57BL/6J) were pulsed with KLH and then injected s.c. into otherwise untreated C57BL/6J mice. These animals were then challenged and examined for footpad swelling responses to KLH. When relatively large numbers (10–50 × 10^3 cells/mouse) of DC were injected, CD95-deficient DC and control DC induced marked DTH reactions at comparable levels (Fig. 13). The extent of swelling response inducible by a single injection of KLH-pulsed DC (5 × 10^3 cells/mouse) was roughly 70% of that induced by immunization with KLH plus CFA. Splenic DC isolated from control mice induced only minimal DTH responses at smaller numbers (0.4–2 × 10^3 cells/mouse). By contrast, splenic DC isolated from the CD95-deficient mice induced maximal responses even at the smallest number (0.4 × 10^3 cells/mouse). Thus, CD95-deficient DC do differ from control DC in their abilities to induce DTH responses in vivo. We interpreted these results to support our hypothesis that CD95/CD95L-mediated, T cell-induced apoptosis of DC is a unique mechanism that prevents the interminable activation of T cells by Ag-bearing DC.

Discussion

As summarized in the Introduction, DC are fully equipped with all phenotypic and functional properties required to initiate cellular immunity. Thus, one would imagine that in the absence of relevant down-regulatory mechanisms, Ag-pulsed DC would continue to present the same Ag to T cells, leading to sustained or excessive immune responses. In fact, several mechanisms have already been reported to suppress DC-induced T cell activation pathway. For example, CD80 and CD86, both of which are expressed by “mature” DC, deliver not only costimulatory signals (via binding to CD28), but also inhibitory signals (via binding to CTLA-4) (56). Repeated activation of T cells triggers fratricidal or suicidal death by a CD95/CD95L-dependent mechanism, a phenomenon known as activation-induced cell death (57–59). More recently, both “myeloid” and “lymphoid” DC subpopulations have been shown to possess the ability to kill alloreactive T cells in a CD95/CD95L-dependent manner (60, 61). These down-regulatory mechanisms all share a common target (i.e., T cells), and they all inhibit the process of clonal expansion of Ag-reactive T cells. Here we report that DC undergo apoptosis soon after Ag-specific interaction with CD4+ T cells, a down-regulatory mechanism that is unique from others in that DC, instead of T cells, serve as the target. Together,

As summarized in the Introduction, DC are fully equipped with all phenotypic and functional properties required to initiate cellular immunity. Thus, one would imagine that in the absence of relevant down-regulatory mechanisms, Ag-pulsed DC would continue to present the same Ag to T cells, leading to sustained or excessive immune responses. In fact, several mechanisms have already been reported to suppress DC-induced T cell activation pathway. For example, CD80 and CD86, both of which are expressed by “mature” DC, deliver not only costimulatory signals (via binding to CD28), but also inhibitory signals (via binding to CTLA-4) (56). Repeated activation of T cells triggers fratricidal or suicidal death by a CD95/CD95L-dependent mechanism, a phenomenon known as activation-induced cell death (57–59). More recently, both “myeloid” and “lymphoid” DC subpopulations have been shown to possess the ability to kill alloreactive T cells in a CD95/CD95L-dependent manner (60, 61). These down-regulatory mechanisms all share a common target (i.e., T cells), and they all inhibit the process of clonal expansion of Ag-reactive T cells. Here we report that DC undergo apoptosis soon after Ag-specific interaction with CD4+ T cells, a down-regulatory mechanism that is unique from others in that DC, instead of T cells, serve as the target. Together,

As summarized in the Introduction, DC are fully equipped with all phenotypic and functional properties required to initiate cellular immunity. Thus, one would imagine that in the absence of relevant down-regulatory mechanisms, Ag-pulsed DC would continue to present the same Ag to T cells, leading to sustained or excessive immune responses. In fact, several mechanisms have already been reported to suppress DC-induced T cell activation pathway. For example, CD80 and CD86, both of which are expressed by “mature” DC, deliver not only costimulatory signals (via binding to CD28), but also inhibitory signals (via binding to CTLA-4) (56). Repeated activation of T cells triggers fratricidal or suicidal death by a CD95/CD95L-dependent mechanism, a phenomenon known as activation-induced cell death (57–59). More recently, both “myeloid” and “lymphoid” DC subpopulations have been shown to possess the ability to kill alloreactive T cells in a CD95/CD95L-dependent manner (60, 61). These down-regulatory mechanisms all share a common target (i.e., T cells), and they all inhibit the process of clonal expansion of Ag-reactive T cells. Here we report that DC undergo apoptosis soon after Ag-specific interaction with CD4+ T cells, a down-regulatory mechanism that is unique from others in that DC, instead of T cells, serve as the target. Together,
multiple mechanisms presumably collaborate to halt DC-induced T cell activation at the levels of both DC and T cells, thereby preventing the interminable activation and excessive expansion of Ag-reactive T cells.

Working with DC isolated from B6.MRL-FAS lpr mice, we have observed that CD95-deficient DC are more potent than wild-type DC in their efficiency to induce DTH responses, especially at lower cell numbers. These results imply that CD95 molecules on DC (either expressed constitutively or induced on activation) have a negative impact on DC-dependent T cell activation in vivo. We have also observed in a series of in vitro experiments that CD95/CD95L interaction plays a functional role in T cell-induced DC apoptosis. Taken together, our observations suggest unique bidirectional signaling pathways during Ag presentation, in which DC deliver activation signals to T cells, whereas the same T cells deliver apoptotic signals to DC. The latter signaling pathway may serve as a mechanism that limits the extent of T cell activation inducible by a given number of Ag-pulsed DC.

In the present study we observed that Ag-specific interaction with the CD4+ T cell clone HDK-1 augments significantly apoptotic cell death of CD11c+ splenic DC in vitro. This corroborates the in vivo observations by Ingulli et al. (27) that OVA-pulsed, fluorescent dye-labeled DC initially interacted closely with OVA-reactive, CD4+ naive T cells in the lymph node but disappeared from this location at later time points. These two sets of observations suggest that DC die after completing the task of Ag presentation to CD4+ T cells. Using a long term DC line XS52, we have addressed the mechanisms of this event: 1) XS52 DC express CD95 on their surface; 2) CD95L expression becomes detectable on HDK-1 T cells after stimulation; 3) ligation of surface CD95...
with anti-CD95 mAb triggers apoptosis of XS52 DC only in the presence of cycloheximide; and 4) neutralizing mAb against CD95L blocks significantly, albeit partially, HDK-1 T cell-induced XS52 DC apoptosis. These observations imply that coupling of CD95 (on DC) with CD95L (on T cells activated by DC) serves as one, but likely not the only, mechanism by which DC undergo apoptosis during Ag presentation. Obviously, further studies, especially at animal levels, will be required to determine the relative contribution of the CD95/CD95L system to T cell-mediated apoptosis of DC.

This is not the first report to describe the potential of DC to undergo apoptosis or to document CD95 expression by DC. Ludewig et al. (62) reported previously that DC undergo spontaneous apoptosis in culture and that this process is augmented by IL-10. We observed previously that ultraviolet B radiation sensitizes DC to become highly susceptible to apoptotic signals delivered by LPS treatment (35). More recently, Chambers et al. (63) found that DC serve as a sensitive target of NK cell-mediated, perforin-dependent cytolysis. Thus, DC undergo apoptosis in response to several different stimuli and by different mechanisms. With respect to CD95 expression, Winzler et al. (64) reported surface expression of CD95 by a long term DC line derived from mouse spleen. CD95L expression was also detected on this splenic DC line, corroborating the report by Süss and Shortman (60) that a subpopulation of splenic DC (i.e., CD8<sup>+</sup> lymphoid DC) express CD95L and trigger apoptosis of alloreactive T cells. Thus, it is reasonable to propose that the CD95/CD95L system, which is now known to be involved in several immunological phenomena (e.g., CTL-mediated killing of target cells, suicidal cell death of T cells, and induction of immunological tolerance) (42), plays two distinct roles in the regulation of DC function.

**FIGURE 10.** Inhibition of T cell-mediated XS52 DC death by anti-CD95L mAb. XS52 cells (1 × 10<sup>5</sup> cells/ml) were incubated for 8 h in the presence or absence of FITC-labeled HDK-1 T cells (4 × 10<sup>5</sup> cells/ml) and/or KLH (100 µg/ml). Anti-CD95L (MFL-1, 10 µg/ml), control hamster IgG, or anti-CD48 (hamster IgG) was added continuously to these cultures. Data in A are representative histograms of PI uptake (FL-2 channel) within the FITC-negative population (i.e., XS52 cells). Data in B are means ± SD (n = 3) of percentage of PI-positive cells within FITC-negative populations. Asterisks indicate statistically significant differences (p < 0.01).
roles in DC-T cell interaction to induce apoptotic cell death of Ag-reactive T cells and Ag-pulsed DC. Other APC populations (e.g., B cells and macrophages) are known to be killed by CD4$^+$ T cells in Ag-specific and CD95/CD95L-dependent manners (43, 44, 50). Likewise, CD54-CD11a/CD18 interaction has been reported to be involved in T cell-mediated apoptosis of B cells (65). Thus, our observations made with DC are in complete agreement with the previous reports with other APC populations. It is to be emphasized that this is the first report formally documenting that DC, the most potent APC population, undergo apoptosis during Ag-specific interaction with CD4$^+$ T cells. Taken together, one may propose that apoptosis occurs commonly in all APC populations after interaction with CD4$^+$ T cells, thereby serving as a mechanism to down-regulate cellular immune responses.

In an attempt to develop strategies to experimentally control T cell-mediated DC apoptosis, we have identified that DC apoptosis is prevented by each of anti-Ia, anti-CD95L, and anti-CD54 mAb. Studies are under way to test the in vivo relevance of these observations. We anticipate that this line of investigation may ultimately lead to the development of new therapeutic reagents to prevent and even treat various human diseases that are caused by excessive or deficient responsiveness to environmental Ag, microbial Ag, tumor-associated Ag, or to self Ag.

**FIGURE 11.** Inhibition of T cell-mediated splenic (Sp) DC death by anti-CD95L mAb. Splenic DC ($1 \times 10^6$ cells/ml) isolated from BALB/c mice were cocultured for 20 h with HDK-1 T cells ($1 \times 10^6$ cells/ml) in the presence or absence of 100 μg/ml KLH. Anti-CD95L (MFL-1, 10 μg/ml), control hamster IgG, or anti-CD48 (hamster IgG) was added continuously to these cultures. The samples were labeled with FITC-conjugated anti-CD11c mAb and then examined for PI uptake. Data are means ± SD ($n = 3$) of percentage of PI-positive cells within FITC-positive populations (i.e., splenic DC). Asterisks indicate statistically significant differences ($p < 0.01$).

**FIGURE 12.** Functional role of CD54 in T cell-mediated apoptosis of XS52 DC. A, XS52 cells and HDK-1 T cells were stained with anti-CD54 mAb, anti-CD11a mAb, or anti-CD18 mAb (filled histograms). Background staining profiles with an isotype-matched control IgG are shown with open histograms. B, FITC-labeled XS52 cells ($1 \times 10^5$ cells/ml) were cocultured with HDK-1 T cells ($4 \times 10^5$ cells/ml) and KLH (100 μg/ml) in the presence of the indicated concentrations of anti-CD54 mAb (●) or an isotype-matched control IgG (○). Samples were then examined for PI uptake. Data are the percentage of inhibition of XS52 DC death (mean ± SD, $n = 3$) as compared with the control in the absence of added Abs.
FIGURE 13. Comparison between CD95-deficient DC vs control DC for their in vivo Ag-presenting capacity. Splenic DC isolated from B6. MRL-FAS<sup>−/−</sup> mice (●) and from control C57BL/6J mice (□) were pulsed with KLH (100 μg/ml) and then injected s.c. into C57BL/6J mice (five mice/group) at the indicated cell numbers. Ten days later, these animals were challenged by s.c. injection of KLH and examined for footpad swelling responses at 24 and 48 h. Data are representative of three independent experiments, showing the means ± SD (n = 5). Asterisks indicate statistically significant differences (p < 0.005). The extent of footpad swelling responses inducible by standard immunization with KLH plus CFA were 19.3 ± 2.3 (n = 5) at 24 h and 17.3 ± 3.8 at 48 h.

References


