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Cutting Edge: Antigen-Specific T Lymphocytes Regulate Lipopolysaccharide-Induced Apoptosis of Dendritic Cells In Vivo[1]

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The potent accessory properties of dendritic cells (DC) develop sequentially during a process termed "maturation." Splenic DC undergo functional maturation in vivo in response to the bacterial product LPS and migrate from the marginal zone to the T cell areas. The redistribution of fully mature DC, which present Ags encountered in the periphery, in the T cell area is likely to result in T cell priming. Unexpectedly, we found that DC rapidly die by apoptosis once they have entered the T cell zone. Injection of OVA peptide in OVA-specific, TCR-transgenic mice strongly delays the LPS-induced apoptosis of DC in situ. We conclude that mature DC are programmed to die unless they receive a survival signal from T cells and that the regulation of DC survival may be a mechanism aimed at controlling the initiation and the termination of the immune response. *The Journal of Immunology,* 1998, 161: 4476–4479.

T he initial steps of the immune response in vivo are still poorly understood. Optimal activation of naive T cells requires at least two signals that are best provided by the same APC: the recognition of the correct Ag/MHC complex by the TCR as well as the interaction of costimulatory molecules with their ligands. These cognate interactions between the APC of the primary response and naive T lymphocytes therefore imply a physical interaction of both cells. Interestingly, dendritic cells (DC)[3] and T cells are typically present at distinct sites of the body, suggesting that priming will require that Ag-bearing DC travel to tissue where responding T cells are found. DC are widely distributed in nonlymphoid and lymphoid organs (1), whereas T cells are located mainly in discrete sites in lymphoid organs. Although both cell types recirculate in the blood and lymph, there is evidence that T/DC interaction occurs in lymphoid organs that are the major sites for the generation of primary immune responses (2, 3).

We have recently shown that bacterial stimuli such as LPS induce the migration of splenic DC from the marginal zone to the T cell area within a few hours (4). The movement of these cells parallels changes in function (4) that allow them to accumulate Ag for later presentation (5, 6). Unexpectedly, the redistribution of DC in T cell area was rapidly followed by a dramatic decrease in the numbers of DC in the spleen (4). The data presented herein show that, starting within hours after LPS-induced maturation, increasing numbers of apoptotic DC accumulate in the T cell area. Considering that colocalization of fully mature DC and T lymphocytes is likely to be the first step of the induction of the immune response in vivo, we have investigated the possibility that T lymphocytes specific for the Ag presented on DC may prevent or at least delay DC death by apoptosis. Our results indeed show that TCR engagement of T lymphocytes increases DC survival in vivo.

Materials and Methods
Mice

BALB/c mice were purchased from IFFA-CREDO (Bruxelles, Belgium). F$_1$(BALB/c × DO11.10) mice were obtained by crossing male DO11.10 TCR-transgenic mice (7) (kindly provided by Dr. Anne O'Garra, DNAX, Palo Alto, CA) with female BALB/c mice. All mice were housed in our own pathogen-free facility.

Reagents and Abs

LPS from *Escherichia coli* was from Difco Laboratories (Detroit, MI). Chicken OVA peptide 323–339 was from Neosystem (Strasbourg, France). The mAbs used were GL1 (rat anti-CD86), N418 (hamster anti-murine CD11c), 7D6 (mouse anti-murine CD3), 2A1 (rat mAb that reacts with granules within the cytoplasm of DCs (8), kindly provided by Dr. R. Steinman), and anti-Bcl-x (Santa Cruz Biotechnology, Santa Cruz, CA).

In vivo treatment

Mice were injected i.v. with 25 μg LPS with or without 50 μg OVA peptide. Some groups received 200 μg anti-CD154 mAbs (9) i.p. 1 day before LPS administration.

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[3] Abbreviations used in this paper: DC, dendritic cells; TUNEL, terminal deoxynucleotidyltransferase-mediated-dUTP nick end labeling.

Immunohistochemistry

Spleens were fixed for 3 days in Immunohistofix (B. Pajak et al., manuscript in preparation) followed by dehydratation in neat acetone for 6 h. Tissues were embedded in Immunohistowax (B. Pajak et al., manuscript in preparation), sectioned at 3–6 μm, deembedded by washing in acetone for 10 min, and transferred to PBS. The endogenous peroxidase activity was neutralized by 3% H_2O_2 in PBS for 30 min, and the slides were stained for apoptosis using the DNA terminal transferase nick-end translation assay (TUNEL; Boehringer, Mannheim, Mannheim, Germany), followed by avidin-biotin-peroxidase complex (Vectastain ABC kit; Vector Laboratories, Burlingame, CA), and revealed with a solution of diaminobenzidine tetrahydrochloride with metal enhancer (DAB tablets, SigmaFAST; Sigma, St. Louis, MO). The slides were incubated in 3% H_2O_2 in PBS to block residual peroxidase activity and incubated in an avidin/biotin blocking kit (Vector Laboratories). The sections were further stained with biotinylated anti-CD11c mAb detected with avidin-biotin-peroxidase complex revealed with 3-amino-9-ethyl-carbazole (Sigma). Sections were mounted in Aquatex (Merck, Darmstadt, Germany). Some sections were sequentially incubated with biotinylated anti-CD3 mAb, avidin-biotin-alkaline phosphatase complex, and the alkaline phosphatase substrate kit III blue (Vector Laboratories). The sections were incubated in an avidin/biotin blocking kit and further stained with TUNEL and DC-specific mAbs (N418 anti-CD11c or 2A1) as described above.

Digitized images were captured using a Ikegami CCD color camera (Ikegami Tsushinki, Tokyo, Japan) and analyzed using CorelDraw 7 software (Corel, Ottawa, Canada).

Flow cytometry

DC were purified using a procedure that avoids culture and adhesion steps. Spleens were digested with collagenase in the presence of EDTA and separated into low and high density fractions on a Nycodenz gradient (Nycomed Pharma, Oslo, Norway (10)). DC were further enriched on a Mini-Macs column using anti-CD11c-coupled microbeads (Miltenyi Biotec, Bergisch-Gladbach, Germany), stained with fluoresceinated N418 or GL1, and analyzed by flow cytometry using a FACScan cytometer (Becton Dickinson, Mountain View, CA).

Western blot analysis of Bcl-x

DC were lysed, and 5 μg of protein from each sample was resolved on a 14% SDS-PAGE gel and transferred to Hybond-C extra membranes (Amersham, Ghent, Belgium). Membranes were saturated overnight, probed with anti-Bcl-x, and detected with horseradish peroxidase-protein A (Sigma) and enhanced chemiluminescence substrate (ECL; Amersham).

Results

Injection of LPS induces apoptosis of DC

We have previously provided evidence (4) that administration of LPS results in early migration of splenic DC to T cell area followed by their physical disappearance. To test whether the loss of DC is caused by apoptotic death, we stained cells on embedded sections of spleens from animals injected with LPS 14 h previously. We have recently developed a new embedding and fixation technique that maintains the cellular morphology and permits membrane and intracellular staining of single cells (section width = 3–6 μM). Apoptotic cells were visualized by the TUNEL reaction. The data in Figure 1 show that TUNEL-positive cells were detected in the spleen of LPS-treated animals (Fig. 1*B*), whereas very few cells were stained in control mice (Fig. 1*A*). Most apoptotic cells expressed the DC markers CD11c (Fig. 1, *B–D*) or 2A1 (Fig. 1*E*) and were localized in the T cell zone around the central arteriole.

Activated T cells increase the survival of DC

To test whether Ag-specific T lymphocytes would increase the survival of DC, we have taken advantage of TCR transgenic mice that have the majority of T cells expressing a TCR specific for OVA/I-A^d. The data in Figure 2 indicate that injection of OVA peptide in TCR-transgenic mice results in the accumulation of mature DC in the T cell area until 24 h after LPS administration, whereas very few DC were detected in the spleen of mice treated
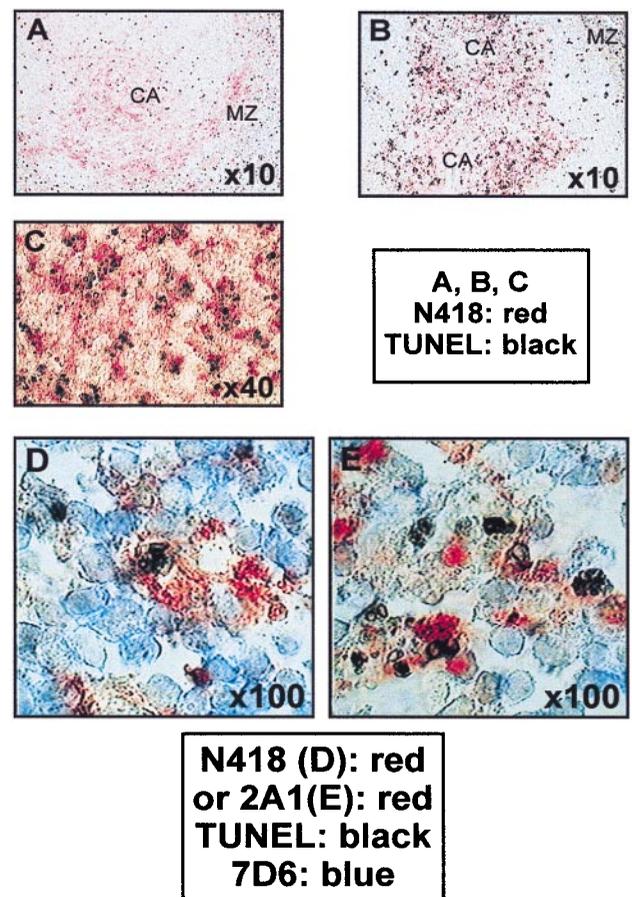

FIGURE 1. Injection of LPS induces apoptosis of splenic DC. Immunostaining of spleen sections of BALB/c mice treated 14 h previously with NaCl (*A*) or 25 μg LPS i.v. (*B–E*). Sections were double stained with N418 and TUNEL (*A–C*) or triple stained with DC-specific (N418 or 2A1), CD3-specific (7D6) mAbs, and TUNEL (*D, E*). The original magnification was 10× (*A, B*), 40× (*C*), and 100× (*D, E*). MZ, marginal zone; CA, central arteriole.

with LPS alone 16 h earlier. Injection of OVA alone resulted in migration of DC but did not induce their loss (not shown). The enhanced viability of mature DC in vivo correlates with a proportional decrease in the incidence of TUNEL-positive cells (Fig. 2) and with the presence of T lymphocytes expressing the early activation markers CD25 and CD69 (data not shown). Injection of anti-CD154/CD40L mAb in DO11.10-transgenic mice did not prevent the survival of DC (Fig. 2*C*). To test whether interaction with T cells in the presence of Ag would further improve the maturation (4) of DC, we compared the expression of costimulatory molecules on DC enriched from mice injected 12 or 20 h earlier with LPS alone or with LPS and OVA peptide. The results in Figure 3 indicate that CD86 molecules were expressed at slightly higher levels and for a longer period on DC from animals injected with LPS and OVA. Collectively, these observations suggest that T lymphocytes regulate the viability, the maturation, and thereby the function of DC in vivo.

The survival of DC in vivo correlates with increased expression of the antiapoptotic Bcl-x molecule

A recent report has emphasized the role of Bcl-x in promoting the survival of mature bone marrow-derived DCs in vitro (11). To test whether Bcl-x expression was modulated in the presence of activated T cells in vivo, we measured its expression by Western blot
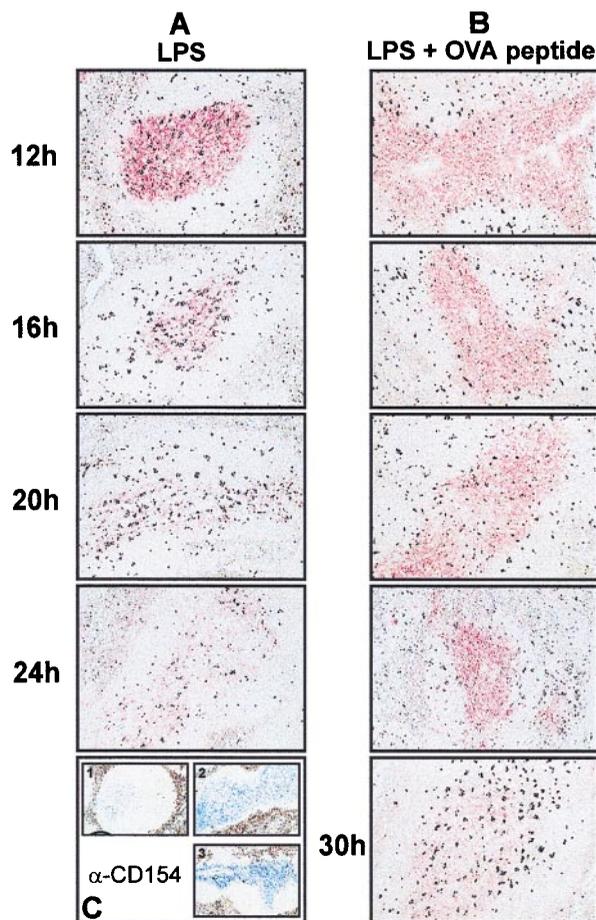
FIGURE 2. Injection of OVA peptide in LPS-treated TCR-transgenic mice increases the survival of DC. *A* and *B*, Immunostaining of spleen sections of F_1(BALB/c × DO11.10) mice treated 12–30 h previously with 25 μg LPS alone (*A*) or in conjunction with 50 μg OVA peptide (*B*). Sections were double stained with N418 (red) and TUNEL (black). *C*, Spleen sections of F_1(BALB/c × DO11.10) mice treated 20 h previously with LPS (1), LPS and OVA peptide (2), or LPS, OVA, and anti-CD154 mAb (3) were stained with N418 (blue). The original magnification was 10×.

FIGURE 4. T cell activation up-regulates Bcl-x in DC in vivo. F_1(BALB/c × DO11.10) received an i.v. injection of NaCl (*lane 1*), LPS (*lane 2*), or LPS and OVA peptide (*lane 3*), and their splenic DC were purified 12 h later. The resulting population contained 90–95% DC as assessed by morphology and flow cytometry using N418 mAb (not shown). Protein (5 μg) extracted from DC was analyzed for Bcl-x protein expression by Western Blot analysis.

These data suggest that activated T lymphocytes could increase DC survival in vivo by regulating their expression of the antiapoptotic Bcl-x molecule.

Discussion

The main finding of this work is that DC that have undergone maturation and migration induced by inflammatory stimuli are programmed to die unless they receive a signal from Ag-specific T cells.

The programmed cell death of DC could be an autonomous mechanism or could be triggered by other cell populations. LPS-induced loss of splenic DC is observed in SCID mice (12) and in beige animals (data not shown), suggesting that T or B lymphocytes are not required and that NK-mediated lysis is not involved in this phenomenon. We favor the hypothesis that DC have discrete stages of life and that mature DC are committed to suicide. Consistent with this hypothesis, three stages of DC maturation (immature, mature, apoptotic) have recently been defined using long-term cultures (13). Of note, our data show that DC that have been rescued following T cell activation still undergo programmed cell death 24–30 h after administration of LPS and OVA peptide. It would be of interest to test whether the delayed disappearance of DC in these transgenic mice results from interruption of the survival signal or from an active mechanism involving the killing of APC by fully activated T cells (14).

The cells and molecules that control the function of DC in vivo are still poorly understood. The data presented herein strongly suggest that T lymphocytes deliver the signal of survival, as injection of OVA in TCR-transgenic mice, but not in BALB/c mice (data not shown), results in delayed apoptosis. There is evidence that CD40 engagement up-regulates MHC and costimulatory molecule expression in vivo in mice and enhances the survival of human DCs in culture (reviewed in Ref. 15). Preliminary results suggest that injection of activating anti-CD40 mAb (3/23; Ref. 16) delays

analysis. DC purified from the spleens of control mice expressed low levels of Bcl-x (Fig. 4). Injection of OVA peptide and LPS resulted in strong up-regulation of Bcl-x expression 12 h after treatment, whereas administration of LPS alone had little effect.
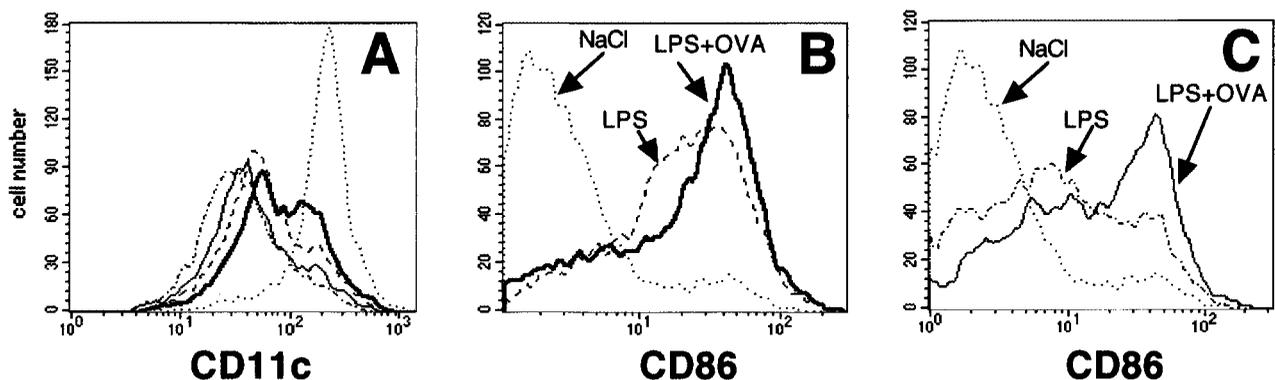

FIGURE 3. Injection of OVA peptide in LPS-treated F_1(BALB/c × DO11.10) mice results in sustained expression of CD86 on DC. DC were purified from mice treated with NaCl, LPS, or LPS and OVA peptide, 12 (*A, B*) or 20 (*A, C*) h previously, and stained for CD11c (*A*) or CD86 (*B, C*) expression. NaCl, dotted line; LPS 12 h, dashed line; LPS + OVA 12 h, bold line; LPS 20 h, dotted/dashed line; LPS + OVA 20 h, thin line.
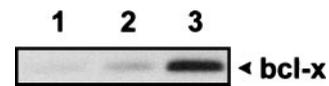
the LPS-induced disappearance of splenic DC (our unpublished observations). However, blockade of CD154/CD40L in DO11.10-transgenic mice does not prevent the survival of DC, suggesting that other receptor/ligand pair(s) or soluble molecules from T lymphocytes may provide a signal of survival. Of interest, a new TNF family member, called TRANCE (TNF-related activation-induced cytokine)/RANKL (receptor activator of NF-κB ligand), has been described that increases the survival and function of DC in vitro (11, 17).

Little is known about the minimal duration of T/DC interaction required for priming in vivo. The Lanzavecchia group has reported that in vitro, naive T cells required ~20 h of sustained signaling to be committed to proliferation (18). In our system, DO11.10 T cells in the presence of the OVA peptide increase the survival of DC by 8–12 h. The persistence of Ag/MHC complexes on surviving DC is indicated by recent reports that immature DC loaded with Ag shortly before maturation display large numbers of stable MHC class II/Ags complexes for long periods (5, 6). Collectively, these observations suggest that the rescue of DC from apoptosis is of physiologic relevance, as it would permit the sustained T/DC interaction required to induce a productive primary T cell response.

T cells and DC appear to reciprocally regulate their life/death. Brocker (19) reported data suggesting that peripheral CD4$^+$ T cells needed MHC class II$^+$ DCs for survival.

Our observations may have important implications for the regulation of the immune response in situ. Apoptosis of new migrant DC that do not interact with Ag-specific T cells would increase the efficiency and selectivity of T/DC interaction in T cell areas of lymphoid organs and of the immune response that ensues. As the frequency of autoimmune T cells that have escaped the negative selection in the thymus is probably lower than the frequency of T lymphocytes specific for non-self Ags, fully mature DCs that present self Ags may preferentially undergo apoptosis. This mechanism could therefore limit the onset of autoimmunity. As the survival of DC would be proportional to the clonal size of Ag-specific T cells, higher numbers of DC may survive during an anamnestic response, thereby increasing the intensity and efficiency of the secondary response. Of note, the delayed programmed cell death of DC that have been rescued by T cell signaling would allow any interacting T cells to disengage and could be an active mechanism provoking the termination of the immune response.

In conclusion, the immune response appears as a dynamic process that involves migration of DC that transport the Ag to the T cell zones of secondary lymphoid organs. In the spleen, immature DC are positioned to filter Ags from the blood in the marginal zone. The movement of DC is associated with phenotypic and functional changes, which allow them to present Ags encountered in the periphery. Mature DC rapidly undergo apoptosis, unless they receive a survival signal from T cells. The induction of maturation by microbial products and the regulation of DC life by T cells should favor the induction of immune responses specific for non-self pathogens.

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