The Journal of Immunology

CD11c$^+$ Dendritic Cells Are Required for Survival in Murine Polymicrobial Sepsis

Philip O. Scumpia, Priscilla F. McAuliffe, Kerri A. O'Malley, Ricardo Ungaro, Takefumi Uchida, Tadashi Matsumoto, Daniel G. Remick, Michael J. Clare-Salzler, Lyle L. Moldawer and Philip A. Efron

J Immunol 2005; 175:3282-3286; ;
doi: 10.4049/jimmunol.175.5.3282
http://www.jimmunol.org/content/175/5/3282

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.

CD11c$^+$ Dendritic Cells Are Required for Survival in Murine Polymicrobial Sepsis[1]

Philip O. Scumpia,* Priscilla F. McAuliffe,* Kerri A. O'Malley,* Ricardo Ungaro,* Takefumi Uchida,* Tadashi Matsumoto,* Daniel G. Remick,‡ Michael J. Clare-Salzler,† Lyle L. Moldawer,[2]* and Philip A. Efron*

CD11c$^+$ dendritic cells (DCs) are APCs that link innate and adaptive immunity. Although DCs are lost from spleen and lymph nodes in sepsis, their role in outcome remains unclear. Transgenic mice (B6.FVB-Tg$^{Itgax\text{-}DTR/EGFP.57}$Lan/J) expressing the diphtheria toxin (DT) receptor on the CD11c promoter (DCKO mice) received 4 ng/kg DT, which resulted in depletion of 88–95% of mature myeloid and lymphoid DCs, with less depletion (75%) of plasmacytoid DCs. Pretreatment of DCKO mice with DT resulted in reduced survival in sepsis compared with saline-pretreated DCKO mice (0 vs 54%; $p < 0.05$) or DT-treated wild-type littermates (0 vs 54%; $p < 0.05$). This increased mortality was not associated with either increased bacteremia or plasma cytokine concentrations. Intravenous injection of 10^7 wild-type DCs improved survival in DCKO mice (42 vs 0%; $p = 0.05$). These data confirm that DCs are essential in the septic response and suggest that strategies to maintain DC numbers or function may improve outcome. *The Journal of Immunology,* **2005, 175: 3282–3286.**

Dendritic cells (DCs)[3] are components of the innate immune system that sense microbial products and initiate adaptive T cell responses (1). Activation of DCs leads to their migration to peripheral lymph nodes, as well as their maturation. This maturation process is characterized by increased secreted cytokine production and surface expression of MHC class I (MHCI) and II (MHCII) and costimulatory molecules, which allows them to present Ag and activate T cells. Defects in this maturation process may lead to either immune suppression or autoimmunity (2).

Despite continuing progress in understanding the pathogenesis of sepsis, interventional therapies have only been able to modestly decrease the associated high morbidity and mortality (3–5). Trauma and sepsis are associated with profound immunological dysfunction, including anergy, and increased apoptosis of various immune cell populations, such as CD4$^+$ lymphocytes, which dramatically alters the host's adaptive response to an invading pathogen (6, 7). Interestingly, antiapoptotic treatments, such as caspase-3 inhibition and overexpression of Bcl-2 in T cells, have been shown to improve outcome in animal models of sepsis (8, 9).

Recently, we reported a profound loss of CD11c$^+$ DCs from spleen and lymph nodes in murine sepsis induced by a cecal ligation and puncture (CLP) (10). Forty to 50% losses of both total (CD11c^{high}) and lymphoid (CD8α^+) DC populations were seen within 24 h. Similar results have been observed by others (11, 12), and Hotchkiss et al. (13) have reported an increased loss of DCs from the spleens of patients who had died from sepsis. Furthermore, the intrapulmonary installation of bone marrow-derived DCs prevented lethal *Aspergillosis* infection in septic animals (14), suggesting a functional role for DCs in opportunistic fungal infections in the lung during sepsis.

The current study was undertaken to determine whether DCs play a determinant role in survival in a mouse model of polymicrobial sepsis. We show in this work that depletion of CD11c^{high}MHCII^{high} DCs dramatically increases mortality to sepsis induced by a CLP. Because DC depletion may transiently affect other cell populations, we performed a reconstitution study with the i.v. administration of wild-type myeloid DCs (mDCs). We show that adoptive transfer with wild-type bone marrow-derived DCs restores survival in this model of polymicrobial sepsis. The increased mortality to sepsis in this model does not appear to be associated with reduced antimicrobial activity or an altered inflammatory response. Rather, these studies suggest that DCs play a direct role in sepsis, and attempts to maintain DC number or function during sepsis may lead to improved outcome.

Materials and Methods

Mice

All experiments were approved by the Institutional Animal Care and Use Committee at the University of Florida College of Medicine before their initiation. Specific pathogen-free B6.FVB-Tg$^{Itgax\text{-}DTR/EGFP.57}$Lan/J (referred to as DCKO) mice were purchased from The Jackson Laboratory. They were maintained in a breeding colony at the University of Florida College of Medicine on standard rodent food and water ad libitum. Mice were genotyped from tail DNA using published primer sequences (15). Age- and sex-matched littermates between 8 and 12 wk of age and not expressing the transgene were used as controls.

Depletion of DCs

To deplete CD11c$^+$ DCs, DCKO mice were treated with an i.p. injection of 4 ng/kg of diphtheria toxin (DT) (15). As controls, DCKO mice received an i.p. injection of saline vehicle, and wild-type littermates were treated

*Department of Surgery and †Department of Pathology, University of Florida, Gainesville, FL 32610; and ‡Department of Pathology, University of Michigan, Ann Arbor, MI 48103

Received for publication April 13, 2005. Accepted for publication June 15, 2005.

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 in part by National Institute of General Medical Sciences Grants R01 GM-63421-05 and R37 GM-40586-18 (to L.L.M.) and National Institute of Diabetes and Digestive and Kidney Diseases Grant R21 DK 063422 (to M.J.C.-S.). P.A.E., and P.F.M. were supported by National Institute of General Medical Sciences T32 Training Grant in Burns and Trauma Research GM-08721-04.

[2] Address correspondence and reprint requests to Dr. Lyle L. Moldawer, Department of Surgery, University of Florida College of Medicine, P.O. Box 100286, Gainesville, FL 32610-0221. E-mail address: moldawer@surgery.ufl.edu

[3] Abbreviations used in this paper: DC, dendritic cell; mDC, myeloid DC; lDC, lymphoid DC; pDC, plasmacytoid DC; MHCII, MHC class II; DT, diphtheria toxin; CLP, cecal ligation and puncture; s, soluble.

0022-1767/05/$02.00

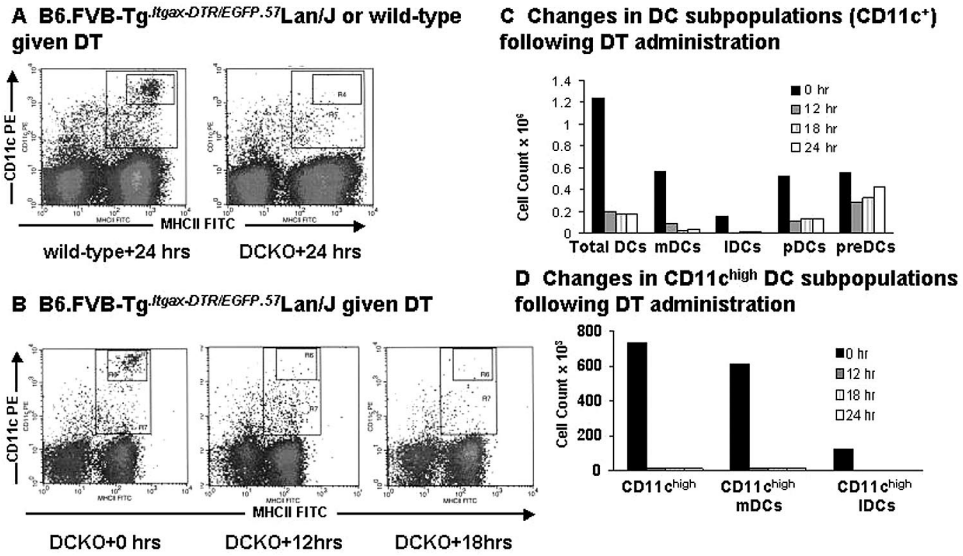

FIGURE 1. Loss of CD11c$^+$ DCs following DT administration. B6.FVB-Tg$^{Itgax\text{-}DTR/EGFP.57}$Lan/J mice either expressing the DT receptor on a CD11c$^+$ promoter (DCKO) or wild-type littermates were administered 4 ng/kg body weight of DT, and splenocytes were obtained at intervals thereafter. After gating out for the presence of debris and dead cells (7-aminoactinomycin D$^+$), living cells were gated for CD11c, MHCII, and specific DC subpopulations, as described in *Materials and Methods*. *A*, Depletion of CD11c$^+$, MHCII$^+$ DCs in DCKO and wild-type littermates 24 h after the administration of DT. *B*, Depletion of CD11c$^+$, MHCII$^+$ DCs in DCKO mice 0, 12, and 18 h after the administration of DT. *C*, Changes in DC subpopulations following DT administration. Total DCs were identified in *A* from the R5 population of CD11c$^+$MHCII$^+$ cells. mDCs were identified as CD11c$^+$CD11b^{high}CD8α^-, lDCs as CD11c$^+$CD8α^+, pDCs as PDCA1$^+$B220$^+$CD11c^{low}CD11b$^-$, and precursor DCs (pre-DCs) as CD11c^{low}MHCII$^-$. DT treatment of DCKO produced a dramatic loss of all DC populations, with a rebound in precursor DCs at 18 and 24 h. *D*, Changes in CD11c^{high} DC populations. CD11c^{high} DC populations were identified in *B* from region R6. DT treatment resulted in the near-complete elimination of both mDC and lDC populations expressing high levels of CD11c.

with DT. Following i.p. injections, mice underwent CLP or were sacrificed for harvesting of spleen and blood.

Cecal ligation and puncture

For induction of polymicrobial sepsis, mice ($n = 11$ per treatment group) were subjected to CLP as previously described (16, 17). In brief, laparotomy was performed, and the cecum was isolated, ligated, and punctured through and through with a 22-gauge needle. When indicated, animals were given either an i.p. injection of DT (4 ng/kg body weight) or an identical volume (200 μl) of sterile normal saline 24 h before the CLP. Also, when indicated, mice received a tail vein injection of 10^7 bone marrow-derived DCs from wild-type animals, or 200 μl of normal saline at the time of DT injection. Depending on the experiment, mice were either euthanized at 6 h after surgery to harvest blood, liver, lungs, and spleen, or animals were observed for up to 7 days to determine survival.

Determination of bacteremia

Blood bacteremia was determined by culturing 10 μl of whole blood diluted with 10 vol of sterile physiologic saline on sheep's blood agar plates (Fisher Scientific) at 37°C in 5% CO_2 for 48 h.

Plasma cytokine concentrations

Plasma cytokine concentrations were determined using a multiplex approach based on Ab recognition of the individual cytokines, as previously described (18). Briefly, capture Abs were spotted on individual nitrocellulose pads affixed to glass slides. Following blocking, the samples were incubated on the slides. Biotinylated secondary Abs and Cy5-conjugated streptavidin were subsequently used for detection. Concentrations were calculated from standard curves.

Culture and i.v. injection of bone marrow-derived DCs

Bone marrow-derived DCs were cultured using the procedures of Lutz et al. (19). Briefly, mice were euthanized, and femurs and tibiae of 6- to 12-wk-old wild-type littermates were removed and cleansed of tissue, and sterilized in 70% ethanol. Both ends of each bone were cut, and marrow was flushed with PBS. RBC were lysed with an ammonium chloride lysis solution, and cells were plated at 2×10^6 per 100-mm petri dish (Falcon; BD Biosciences) in 10 ml of RPMI 1640 medium, supplemented with penicillin, streptomycin, 2-ME, and 10% heat-inactivated and filtered FCS. rmGM-CSF (200 U/ml; PeproTech) was added to the plates and again at 3 days of culture. On day 6, one-half of the cell-containing supernatant was collected, centrifuged, and resuspended in 10 ml of fresh medium containing GM-CSF. On day 8, nonadherent and loosely adherent cells were harvested, washed in PBS, and collected into a syringe at 5×10^6 cells per 100 μl. Then, 200 μl was injected i.v. into each mouse at the time of DT administration, 24 h before the CLP.

Phenotypic analysis of DC populations

Spleens were harvested 12, 18, or 24 h after DT injection, and single-cell suspensions were created by passing the cells through a 70-μm pore size cell strainer (Falcon). Contaminating erythrocytes were lysed with an ammonium chloride lysis solution. After washing twice with buffer (1% BSA and 1 mM EDTA (Fisher)) and 0.1% sodium azide (NaN_3; Sigma-Aldrich) in HBSS without phenol red, calcium, and magnesium (Cellgro; Mediatech), cells were resuspended in 4% Hanks' azide buffer (HBSS without calcium, magnesium, or phenol red, with 4% BSA, 0.1% sodium azide, 0.2% anti-CD16/32, and 1 mM EDTA), and then stained. All Abs were purchased from BD Pharmingen except the anti-plasmacytoid DC Ag-1 (PDCA-1) Ab, which was purchased from Miltenyi Biotec. DCs were characterized using anti-I/A-I/E (MHCII) conjugated to FITC, anti-CD11c conjugated to PE, anti-CD8 conjugated to FITC or allophycocyanin (clone 53-6.7), and anti-CD11b-FITC or -allophycocyanin, or anti-PDCA-1-PE, and anti-B220-allophycocyanin Abs. The maturation status of the CD11c$^+$ DCs was assessed by the level of MHCII expression. Samples were acquired and analyzed on a FACSCalibur flow cytometer (BD Biosciences). mDCs were characterized as CD11c$^+$MHCII$^+$CD11b^{high}CD8α^-, lymphoid DCs (lDCs) were characterized as CD11c$^+$MHCII$^+$CD8α^{high}, and plasmacytoid DCs (pDCs) were characterized as PDCA-1$^+$B220$^+$CD11c^{low} or PDCA-1$^+$B220$^+$CD11b$^-$. Precursor DCs were characterized as CD11c$^+$MHCII$^-$ cells. At least 3×10^4 nondebris, live cells (7-aminoactinomycin D$^-$) were used for analysis.

Statistics

Differences in survival were determined by the Fisher exact test. Continuous variables were first tested for normality and equality of variances. Differences among groups were evaluated by ANOVA, with post hoc comparisons performed using Dunn's multiple range test. Significance was determined at the 95% confidence level.

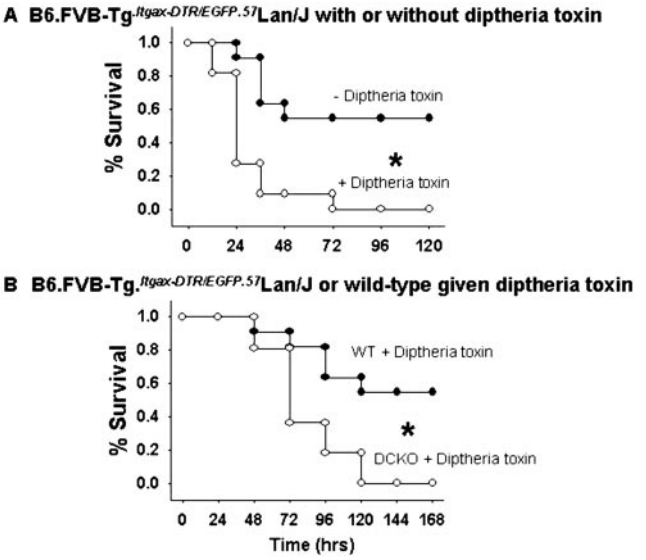

FIGURE 2. DCKO, but not wild-type, mice are more susceptible to lethality following CLP after DT administration. *A*, Survival was determined in DCKO ($n = 11$) vs wild-type littermates ($n = 11$) following the administration of 4 ng/kg DT 24 h before the induction of CLP. Survival was improved in wild-type littermates vs the DCKO (*, $p < 0.05$). *B*, Survival following CLP was markedly reduced in DCKO mice ($n = 11$) pretreated with DT, compared with wild-type mice also receiving DT (*, $p < 0.05$).

Results

DT administration depletes CD11c^{high}MHCII^{high} DCs in B6.FVB-Tg.$^{Itgax\text{-}DTR/EGFP.57}$Lan/J mice

The transgenic B6.FVB-Tg.$^{Itgax\text{-}DTR/EGFP.57}$Lan/J mice express the DT receptor on the CD11c promoter, which allows for selective depletion of DCs following the administration of DT. In these mice, a single injection of DT did not produce significant morbidity or mortality. The only physiologic response noted was delayed awakening in response to the anesthesia. We first verified that DT causes depletion of DCs from the spleen of DCKO mice. As shown in Fig. 1*A*, 24 h after DT administration, spleens were depleted of predominantly CD11c^{high}MHCII^{high} DCs and to a lesser extent CD11c^{low} DCs. The loss began by 12 h and was sustained for at least 24 h (Fig. 1*B*). Next, we determined the kinetics and subtypes of CD11c$^+$ DC loss following DT administration. We found that the nadir in CD11c^{high}MHCII^{high} DCs was 24 h after DT administration, regardless of DC subtype. The CD11c^{high} lDC (CD8α^+) subtype was depleted most by DT, and demonstrated a 95% loss by 24 h (Fig. 1*D*). CD11c^{high} mDCs (CD11b^{high}CD8α^-) were also dramatically lost (88–90%) by 24 h, whereas pDCs (PDCA-1$^+$B220$^+$CD11c$^{low/int}$CD11b$^-$), which are intermediate expressers of CD11c, demonstrated only 75% loss by 24 h (Fig. 1, *C* and *D*). Interestingly, by 24 h after DT administration, a CD11c^{low}MHCII$^-$ DC (pre-DC) population began to appear in the spleen (Fig. 1*C*). These cells could be recent bone marrow emigrants that are DC precursors but may become susceptible to DT as they begin to express higher levels of CD11c.

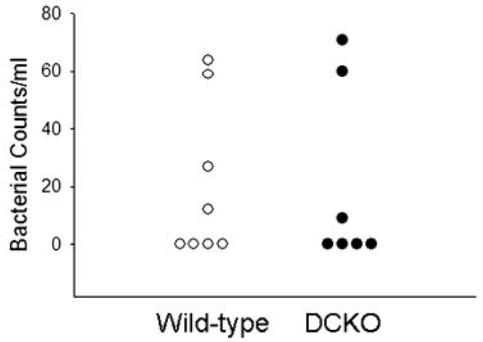

FIGURE 3. CLP induces blood bacteremia in DCKO and wild-type mice treated with DT. DCKO ($n = 7$) and wild-type mice ($n = 8$) were subjected to CLP 24 h after administration of 4 ng/kg DT. Six hours following CLP, 100 μl of whole blood was aseptically plated on sheep blood agar plates, and placed in an incubator (37°C, 5% CO_2). Total aerobic bacterial colonies were determined 24 h after plating. Blood from sham-treated DCKO and wild-type mice (0 of 3 and 0 of 3) did not yield positive bacterial culture (data not shown).

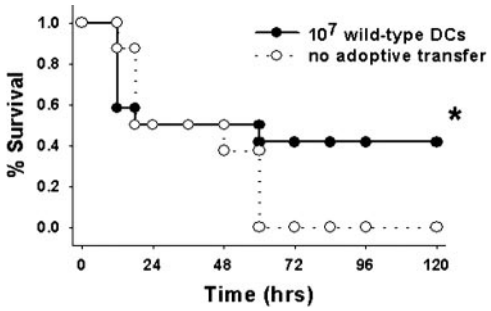

FIGURE 4. Adoptive transfer of 10^7 bone marrow-derived wild-type DCs prevents CLP-induced mortality in DCKO mice pretreated with DT. DCKO mice were pretreated with 4 ng/kg DT 24 h before CLP. At the time of pretreatment, animals either received the i.v. administration of 10^7 DCs obtained from wild-type littermates ($n = 12$) or an identical volume of normal saline ($n = 8$). Mice that received the i.v. adoptive transfer with wild-type DCs had improved outcome after CLP (*, $p < 0.05$ by Fisher exact test).

DCKO mice are more susceptible to mortality following CLP

To determine whether loss of CD11c$^+$ DCs increases mortality after CLP, DCKO mice underwent CLP 24 h after DT or saline administration. As shown in Fig. 2*A*, DCKO mice pretreated with DT displayed increased mortality after CLP compared with DCKO mice pretreated with saline (100 vs 45%; $p < 0.05$). Although wild-type mice do not express the DT receptor, we wanted to assure that DT had no direct effect on survival after CLP in littermates not expressing the CD11c$^+$-DT receptor transgene. Fig. 2*B* shows that additional DCKO mice treated with DT displayed increased mortality compared with their littermates not expressing the DT receptor, but also treated with DT, following CLP, showing that DT has no effect on wild-type littermates (100 vs 45%; $p < 0.05$).

DCKO mice do not display increased bacteremia or altered cytokine production following CLP

Because DCKO mice begin to display significant mortality earlier than 12 h following the CLP, we examined whether the increased mortality was associated with an increased blood bacteremia, or an exaggerated inflammatory response with associated organ injury. If there were reduced immune surveillance associated with the loss of CD11c$^+$ DCs, it might present as increased systemic bacteremia derived from the release of luminal contents. We found that blood from sham-treated healthy mice did not yield positive bacterial culture ($n = 3$ in each group), and that there was no difference between the numbers of mice that developed bacteremia 6 h after CLP in the DCKO and wild-type littermates treated with DT (3 of 7 vs 4 of 8, respectively; $p > 0.05$). In those mice that exhibited

Table I. *Plasma cytokine concentrations following CLP*[a]

	sTNFR1 (pg/ml)	IL-1ra (pg/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	IL-13 (pg/ml)	IL-18 (pg/ml)	IL-12 p70 (pg/ml)	IFN-γ (pg/ml)	MCP-1 (pg/ml)	MIP-2 (pg/ml)
Sham, wild-type littermates + DT	222 ± 192	2684 ± 2705	59 ± 6	12 ± 0	147 ± 213	12 ± 0	12 ± 0	12 ± 0	188 ± 76	24 ± 0
Sham, DCKO mice + DT	479 ± 217	1854 ± 741	1875 ± 2598	12 ± 0	83 ± 71	67 ± 95†	12 ± 0	12 ± 0	291 ± 211	225 ± 348
Wild-type littermates + DT following CLP	999 ± 178*	55089 ± 52935*	15806 ± 7956*	664 ± 830	244 ± 239	19 ± 19	12 ± 0.8	72 ± 158	1949 ± 1779*	4818 ± 4258*
DCKO mice + DT following CLP	1082 ± 356*	31547 ± 27092*	18181 ± 12607*	365 ± 280	328 ± 285	267 ± 185†	25 ± 22	21 ± 18	1602 ± 1020*	3027 ± 2403*

[a] Values represent the mean ± SD.
*, $p < 0.05$, CLP vs sham; †, $p < 0.05$, DCKO vs wild-type littermates.

bacteremia, there was also no gross difference in the numbers of aerobic bacteria recovered from the blood (Fig. 3).

We next assessed whether the increased mortality after CLP in DCKO mice was associated with an exaggerated inflammatory response. Our concern in this case was that the loss of these DCs might prime these animals for a subsequent inflammatory response to the CLP. As shown in Table I, there were dramatic increases in the plasma concentrations of a number of inflammatory cytokines 6 h after the CLP. These included soluble (s)TNFR1, sTNFR2, IL-1ra, IL-6, MCP-1, and MIP-2, as well as IL-1β, lipopolysaccharide-induced CXC chemokine, eotaxin, and MIP-1α (all $p <$ 0.05 by ANOVA). No differences in the plasma concentrations of IL-4, IL-5, IL-10, IL12p70, IL-13, IFN-γ, RANTES, or TNF-α were found between any groups ($p > 0.05$ by ANOVA). Also, there were no differences in the concentration of any individual cytokine in DCKO pretreated with DT vs wild-type littermates pretreated with DT after sham or CLP, with the exception that IL-18 concentrations were markedly higher in the DCKO mice ($p < 0.05$ by ANOVA).

Treatment with wild-type bone marrow-derived DCs protects DCKO mice from mortality in CLP

We next examined whether the loss of DCs is directly responsible for the increased mortality in the DCKO mice. We have previously characterized the DCs generated for use in this experiment and found that they are a mixture of $CD11c^{high}MHCII^{high}$ (37–39%) and $CD11c^{low}MHCII^{low}$ DCs (20). We found that i.v. injection of 10^7 bone marrow-derived DCs at the time of DT injection protected DCKO mice from mortality. Mortality declined from 100 to 65% in DCKO mice reconstituted by the i.v. administration of DCs obtained from wild-type littermates ($p < 0.05$) (Fig. 4).

Discussion

The treatment of sepsis remains a clinical conundrum, in part because our understanding of the immunological basis for the progression of sepsis to organ injury and mortality remains incomplete (21). The alterations in the innate and acquired immune system produced by severe sepsis are dramatic, and include profound losses of $CD4^+$ T cells, $CD11c^+$ DCs, and B cells, but not NK cells or macrophages from peripheral lymph nodes and spleen (6, 7, 10, 12).

The functional significance of this loss of T cells and DCs from secondary lymphoid organs during the sepsis response has been the subject of considerable controversy. The loss of both $CD4^+$ T cells and DCs appears to be associated with an increase in their cellular apoptosis, which has proven to be an attractive but unproven therapeutic target in sepsis (22). Treatment of septic mice with caspase-3 inhibitors prevents mortality, although these studies could not identify the cellular target for the inhibition of apoptosis (8). We and others have shown that improved survival to severe sepsis induced by CLP could be seen in transgenic mice whose lymphocytes were overexpressing the antiapoptotic protein BCL-2 (8, 23, 24). In these animals, the increased loss of apoptotic T cells was prevented, and mortality was subsequently reduced.

Unfortunately, similar findings have not been previously seen with $CD11c^+$ DC populations in bacterial sepsis. However, there is a body of circumstantial evidence to suggest that $CD11c^+$ DC function is required for a successful outcome to sepsis. For example, depletion of $CD11c^+$ DCs has a significant impact on the natural course of infections or inflammation. In the original description of these DCKO mice, Jung et al. (15) observed that $CD11c^+$ DC depletion resulted in the abrogation of CTL responses to *Listeria* and *Plasmodium* infections. In a more recent finding, van Rijt et al. (25) observed that DCKO mice could not respond with an appropriate Th2 response to airway inflammation.

Alternatively, we have demonstrated that modifying DC phenotype can also have a substantial impact on outcome from bacterial sepsis. In two previous reports, we observed that the in vivo transfection of DCs with an adenoviral recombinant expressing IL-10 generated a regulatory-like DC population that protected mice from CLP-induced mortality (17, 23). Interestingly, similar improvements in outcome could be obtained when the animals received adoptive transfer of DCs transduced ex vivo to produce IL-10 (26). These modified regulatory DCs, when readministered into the footpads of mice, were shown to traffic to the draining lymph nodes, but not to the spleen. Such findings suggest that DC function can play a central role in determining outcome to severe sepsis, and modifying either the number or phenotype of these cells can impact outcome from severe sepsis.

The question that naturally arises from these studies is the following: what are the mechanisms by which the loss of $CD11c^+$ DCs results in such dramatic reductions in survival? The one-time administration of DT to these transgenic mice results in the necrotic and apoptotic cell death of DCs in organs of the reticuloendothelial system with no direct effect on mortality by itself. We were concerned that the presence of necrotic cells might serve as an endogenous "danger signal" for the innate immune system (27), and induce an exaggerated inflammatory response by tissue macrophages or other inflammatory cells that moderate the inflammatory response to sepsis. To address this concern, we examined the inflammatory cytokine responses in these mice to CLP with the expectation that the magnitude of the proinflammatory response would be heightened in DCKO mice, if their innate immune system had been primed by the presence of dying or necrotic $CD11c^+$ cells. As shown in Table I, CLP was associated with dramatic changes in the plasma concentrations of a number of proinflammatory cytokines in the septic mice 6 h after the induction of CLP. Although there were changes in the concentrations of several

proinflammatory cytokines, the differences in concentrations between wild-type littermates and the DCKO were very modest, with only IL-18 concentrations differing. Thus, it appears unlikely that the increased mortality seen in the DCKO mice was due to an overall exaggeration in the proinflammatory response.

An alternative explanation is that the loss of DCs in these animals reduced the antimicrobial capacity and resulted in a more rapid and complete dissemination of the microbial pathogens. To assess the overall antimicrobial response in the DCKO mice, we examined blood bacteremia at 6 h following CLP. There was no gross difference in either the frequency or magnitude of aerobic bacteremia in the DCKO mice when compared with their wild-type littermates. Although examining a larger group may have yielded significant differences between the groups, it is unlikely that the small differences in bacterial clearance would explain the dramatic discrepancies in mortality.

The question of underlying mechanism(s) remains unresolved, although it is clear that the reductions in survival can be directly attributed to the loss of these CD11c expressing cell populations. Alterations in innate and acquired immunity produced by the cell death of the CD11c-expressing cells cannot explain the differences in outcome, because adoptive transfer with bone-marrow derived DCs obtained from wild-type mice protected animals from lethality. Because these DCKO mice lose significant numbers of their myeloid, lymphoid, and to a lesser extent, their pDCs simultaneously, the contribution that these individual cell populations make to outcome remains unclear. Further studies will be required to dissect the contributions and underlying mechanisms of the individual $CD11c^+$ populations to outcome from sepsis. However, the studies have unequivocally demonstrated for the first time that normal numbers of $CD11c^+$ DCs are required for a successful outcome from severe sepsis.

Disclosures

The authors have no financial conflict of interest.

References

1. Efron, P., and L. L. Moldawer. 2003. Sepsis and the dendritic cell. *Shock* 20: 386–401.
2. Steinman, R. M., D. Hawiger, and M. C. Nussenzweig. 2003. Tolerogenic dendritic cells. *Annu. Rev. Immunol.* 21: 685–711.
3. Abraham, E., A. Anzueto, G. Gutierrez, S. Tessler, G. San Pedro, R. Wunderink, A. Dal Nogare, S. Nasraway, S. Berman, R. Cooney, et al. 1998. Double-blind randomised controlled trial of monoclonal antibody to human tumour necrosis factor in treatment of septic shock: NORASEPT II Study Group. *Lancet.* 351: 929–933.
4. Fisher, C. J., Jr., J. F. Dhainaut, S. M. Opal, J. P. Pribble, R. A. Balk, G. J. Slotman, T. J. Iberti, E. C. Rackow, M. J. Shapiro, R. L. Greenman, et al. 1994. Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome: results from a randomized, double-blind, placebo-controlled trial: Phase III rhIL-1ra Sepsis Syndrome Study Group. *J. Am. Med. Assoc.* 271: 1836–1843.
5. Bernard, G. R., J. L. Vincent, P. F. Laterre, S. P. LaRosa, J. F. Dhainaut, A. Lopez-Rodriguez, J. S. Steingrub, G. E. Garber, J. D. Helterbrand, E. W. Ely, and C. J. Fisher, Jr. 2001. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N. Engl. J. Med.* 344: 699–709.
6. Hotchkiss, R. S., P. E. Swanson, J. P. Cobb, A. Jacobson, T. G. Buchman, and I. E. Karl. 1997. Apoptosis in lymphoid and parenchymal cells during sepsis: findings in normal and T- and B-cell-deficient mice. *Crit. Care Med.* 25: 1298–1307.
7. Hiramatsu, M., R. S. Hotchkiss, I. E. Karl, and T. G. Buchman. 1997. Cecal ligation and puncture (CLP) induces apoptosis in thymus, spleen, lung, and gut by an endotoxin and TNF-independent pathway. *Shock* 7: 247–253.
8. Hotchkiss, R. S., K. W. Tinsley, P. E. Swanson, K. C. Chang, J. P. Cobb, T. G. Buchman, S. J. Korsmeyer, and I. E. Karl. 1999. Prevention of lymphocyte cell death in sepsis improves survival in mice. *Proc. Natl. Acad. Sci. USA* 96: 14541–14546.
9. Hotchkiss, R. S., P. E. Swanson, C. M. Knudson, K. C. Chang, J. P. Cobb, D. F. Osborne, K. M. Zollner, T. G. Buchman, S. J. Korsmeyer, and I. E. Karl. 1999. Overexpression of Bcl-2 in transgenic mice decreases apoptosis and improves survival in sepsis. *J. Immunol.* 162: 4148–4156.
10. Efron, P. A., A. Martins, D. Minnich, K. Tinsley, R. Ungaro, F. R. Bahjat, R. Hotchkiss, M. Clare-Salzler, and L. L. Moldawer. 2004. Characterization of the systemic loss of dendritic cells in murine lymph nodes during polymicrobial sepsis. *J. Immunol.* 173: 3035–3043.
11. Tinsley, K. W., M. H. Grayson, P. E. Swanson, A. M. Drewry, K. C. Chang, I. E. Karl, and R. S. Hotchkiss. 2003. Sepsis induces apoptosis and profound depletion of splenic interdigitating and follicular dendritic cells. *J. Immunol.* 171: 909–914.
12. Ding, Y., C. S. Chung, S. Newton, Y. Chen, S. Carlton, J. E. Albina, and A. Ayala. 2004. Polymicrobial sepsis induces divergent effects on splenic and peritoneal dendritic cell function in mice. *Shock.* 22: 137–144.
13. Hotchkiss, R. S., K. W. Tinsley, P. E. Swanson, M. H. Grayson, D. F. Osborne, T. H. Wagner, J. P. Cobb, C. Coopersmith, and I. E. Karl. 2002. Depletion of dendritic cells, but not macrophages, in patients with sepsis. *J. Immunol.* 168: 2493–2500.
14. Benjamim, C. F., S. K. Lundy, N. W. Lukacs, C. M. Hogaboam, and S. L. Kunkel. 2004. Reversal of long-term sepsis-induced immunosuppression by dendritic cell. *Blood* 105: 3588–3595.
15. Jung, S., D. Unutmaz, P. Wong, G. Sano, K. De los Santos, T. Sparwasser, S. Wu, S. Vuthoori, K. Ko, F. Zavala, et al. 2002. In vivo depletion of $CD11c^+$ dendritic cells abrogates priming of $CD8^+$ T cells by exogenous cell-associated antigens. *Immunity* 17: 211–220.
16. Baker, C. C., I. H. Chaudry, H. O. Gaines, and A. E. Baue. 1983. Evaluation of factors affecting mortality rate after sepsis in a murine cecal ligation and puncture model. *Surgery* 94: 331–335.
17. Oberholzer, A., C. Oberholzer, K. S. Bahjat, R. Ungaro, C. L. Tannahill, M. Murday, F. R. Bahjat, Z. Abouhamze, V. Tsai, D. LaFace, et al. 2002. Increased survival in sepsis by in vivo adenovirus-induced expression of IL-10 in dendritic cells. *J. Immunol.* 168: 3412–3418.
18. Knight, P. R., A. Sreekumar, J. Siddiqui, B. Laxman, S. Copeland, A. Chinnaiyan, and D. G. Remick. 2004. Development of a sensitive microarray immunoassay and comparison with standard enzyme-linked immunoassay for cytokine analysis. *Shock* 21: 26–30.
19. Lutz, M. B., N. Kukutsch, A. L. Ogilvie, S. Rossner, F. Koch, N. Romani, and G. Schuler. 1999. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J. Immunol. Methods* 223: 77–92.
20. Efron, P. A., F. R. Bahjat, R. Ungaro, J. Debernardis, C. L. Tannahill, H. V. Baker, C. K. Edwards, and L. L. Moldawer. 2005. Characterization of the maturation of murine bone-marrow derived dendritic cells with lipopolysaccharide and tumor necrosis factor-a. *J. Endotoxin Res.* 11: 145–160.
21. Oberholzer, A., C. Oberholzer, and L. L. Moldawer. 2001. Sepsis syndromes: understanding the role of innate and acquired immunity. *Shock* 16: 83–96.
22. Oberholzer, C., A. Oberholzer, M. Clare-Salzler, and L. L. Moldawer. 2001. Apoptosis in sepsis: a new target for therapeutic exploration. *FASEB J.* 15: 879–892.
23. Oberholzer, C., A. Oberholzer, F. R. Bahjat, R. M. Minter, C. L. Tannahill, A. Abouhamze, D. LaFace, B. Hutchins, M. J. Clare-Salzler, and L. L. Moldawer. 2001. Targeted adenovirus-induced expression of IL-10 decreases thymic apoptosis and improves survival in murine sepsis. *Proc. Natl. Acad. Sci. USA* 98: 11503–11508.
24. Iwata, A., V. M. Stevenson, A. Minard, M. Tasch, J. Tupper, E. Lagasse, I. Weissman, J. M. Harlan, and R. K. Winn. 2003. Overexpression of Bcl-2 provides protection in septic mice by a *trans* effect. *J. Immunol.* 171: 3136–3141.
25. van Rijt, L. S., S. Jung, A. Kleinjan, N. Vos, M. Willart, C. Duez, H. C. Hoogsteden, and B. N. Lambrecht. 2005. In vivo depletion of lung $CD11c^+$ dendritic cells during allergen challenge abrogates the characteristic features of asthma. *J. Exp. Med.* 201: 981–991.
26. Oberholzer, A., C. Oberholzer, P. A. Efron, P. O. Scumpia, T. Uchida, K. S. Bahjat, R. Ungaro, C. L. Tannahill, M. Murday, F. R. Bahjat, et al. 2005. Functional modification of dendritic cells with recombinant adenovirus encoding IL-10 for the treatment of sepsis. *Shock* 23: 507–515.
27. Matzinger, P. 2002. The danger model: a renewed sense of self. *Science* 296: 301–305.