The Journal of Immunology

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Increased Survival in Sepsis by In Vivo Adenovirus-Induced Expression of IL-10 in Dendritic Cells[1]

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The dendritic cell (DC) is the most potent APC of the immune system, capable of stimulating naive T cells to proliferate and differentiate into effector T cells. Recombinant adenovirus (Adv) readily transduces DCs in vitro allowing directed delivery of transgenes that modify DC function and immune responses. In this study we demonstrate that footpad injection of a recombinant Adv readily targets transduction of myeloid and lymphoid DCs in the draining popliteal lymph node, but not in other lymphoid organs. Popliteal DCs transduced with an empty recombinant Adv undergo maturation, as determined by high MHC class II and CD86 expression. However, transduction with vectors expressing human IL-10 limit DC maturation and associated T cell activation in the draining lymph node. The extent of IL-10 expression is dose dependent; transduction with low particle numbers (10^5) yields only local expression, while transduction with higher particle numbers (10^7 and 10^{10}) leads additionally to IL-10 appearance in the circulation. Furthermore, local DC expression of human IL-10 following in vivo transduction with low particle numbers (10^5) significantly improves survival following cecal ligation and puncture, suggesting that compartmental modulation of DC function profoundly alters the sepsis-induced immune response. *The Journal of Immunology,* 2002, 168: 3412–3418.

Dendritic cells (DCs)[3] are potent APC, distinguished by their ability to induce naive T cells to proliferate and differentiate into effector T cells in the absence of exogenous stimuli (1–3). Immature DCs exist in all peripheral tissues, where they phagocytose and process soluble and particulate Ag. After exposure to an activating stimulus, such as LPS, IL-1α, CpG motifs, or apoptotic cell fragments, the immature DC will begin a maturation process involving modulation of chemokine receptor expression, migration to the draining lymph node, increased processing and presentation of peptides on both class I and class II MHC molecules, up-regulation of costimulatory molecules such as CD80 and CD86, and increased production of soluble cytokines such as IL-12 (3, 4). Within the lymph node, the mature DC secretes chemokines such as macrophage-inflammatory protein-3β to attract large numbers of naive T cells to the area, allowing the presented peptide:MHC to interact with a wide variety of TCR complexes.

Dysregulation of the immune response occurs during sepsis and often leads to the development of multiorgan failure. Although the pathogenesis of the sepsis response to bacterial pathogens has been extensively studied, the mechanisms leading to high morbidity and mortality in patients remain unclear. Possible explanations include an unregulated production of pro- and anti-inflammatory cytokines that leads to endothelial injury as well as increased activation-induced cell death (apoptosis) of T and B lymphocytes that limits acquired immune response-mediated clearance of pathogens (5, 6).

The effects of sepsis on DC function, however, are largely unknown. Bacteremia, endotoxemia, and generalized peritonitis, all of which promote extensive inflammatory cytokine release (e.g., TNF-α) may induce unregulated and widespread activation and maturation of DCs. In the latter phases of sepsis, DCs may globally lose their ability to produce IL-12 and generate protective Th1 immune responses against the primary and secondary infectious organisms (7, 8). Alternatively, extensive activation of DCs at early time points could promote responses that limit the capacity of these cells to contain the sepsis response. Controlling the extent of the inflammatory response and DC activation may provide novel therapeutic approaches to limit the untoward outcomes of the sepsis response.

The goal of the present study was to determine whether modification of DCs toward a less activated state would influence the outcome of sepsis. Previous studies demonstrated that IL-10 potently inhibits DC maturation, Ag presentation, and IL-12 production and maintains macropinocytosis and endocytosis (9, 10). Furthermore, DCs treated with an anti-IL-10 Ab show an increased capacity to activate and prime naive T cells to a more prominent Th1 polarization, suggesting that autocrine regulation of DCs by this cytokine is important (11).

We hypothesized that transduction of DCs to induce transient expression of human IL-10 (hIL-10) would inhibit DC maturation in the setting of sepsis and might modify the outcome to the septic response. To accomplish this, we considered the use of recombinant viral vectors. DCs have been shown recently to be permissive to adenovirus (Adv) infection in vitro at high particle concentrations (12). Due to the relatively short duration of transgene expression, Adv

appears promising as a vector for transient gene therapy (13, 14), appropriate for acute inflammatory processes such as sepsis.

We show in this work that the footpad injection of adenoviral vectors results in efficient transgene expression by DCs in the draining popliteal lymph node but not in other lymphoid organs. Transduction of DCs with adenoviral vectors expressing IL-10 is also associated with reduced DC maturation and reduced $CD4^+$ and $CD8^+$ T cell activation in the draining lymph node. IL-10 gene expression localized to the popliteal lymph node DCs improves the survival of mice challenged with cecal ligation and puncture in a murine model of polymicrobial sepsis. These studies suggest that compartmentalized expression of IL-10 in DCs is effective in improving the outcome in sepsis.

Materials and Methods

Mice

Specific pathogen-free female C57BL/6 mice between 5 and 8 wk of age were purchased from The Jackson Laboratory (Bar Harbor, ME). They were maintained on standard rodent food and water ad libitum. The studies were approved by the institutional animal care and use committee at University of Florida College of Medicine before initiation of these studies.

Construction of a recombinant Adv expressing hIL-10 or gfp

A derivative of human Adv serotype 5 was used as the source of viral DNA backbone. This modified adenoviral vector backbone contains a deletion of bp 355–4,021, resulting in a deletion of the E1a, E1b, and protein IX polypeptides. In addition, there is a deletion of bp 28,592–30,470, which results in a loss of 1.9 kb of DNA from the E3 region. A recombinant Adv expressing the hIL-10 cDNA transgene was constructed using standard homologous recombination methods, as originally described by Graham and Prevec (15). Briefly, the hIL-10 cDNA containing the full-length translated region (from pDSRG-IL10 plasmid, respectively; obtained from K. Moore, DNAX Research Institute, Palo Alto, CA) was subcloned into the *Bam*HI/*Xba*I cloning site of the pACN transfer plasmid. The pACN transfer plasmid is based on pBR322 and contains, from 5′ to 3′, the Adv serotype 5 (Ad5) inverted terminal repeat and packaging signal (Ad5 bp 1–358), the human CMV immediate early enhancer/promoter (CMV), the Adv serotype 2 tripartite leader sequence (PL), a multiple cloning site, and Adv serotype 2 bp 4,021–10,457. This plasmid was cotransfected into 293 cells along with a *Cla*I-linearized fragment of the plasmid described above containing the modified human Adv serotype 5 vector backbone. Additionally, a recombinant Adv containing an empty expression cassette was constructed for use as a control. All viral constructs were similar, with the exception of the transgene, and the production and purification procedures were identical (16).

Footpad gene therapy

Following anesthesia mice were injected into both hind footpads with 50 μl Adv or buffer using an insulin syringe and a 29-gauge needle (BD Biosciences, Franklin Lakes, NJ). Mice received injections of 2×10^5, 2×10^7, or 2×10^{10} particles of a recombinant Adv construct expressing hIL-10 (Adv/hIL-10), green fluorescent protein (gfp) as a reporter gene (Adv/gfp), or an identical Adv recombinant containing an empty expression cassette (Adv/empty) in buffer (PBS supplemented with 2 mM MgCl$_2$ and 3% sucrose). Twenty-four hours later mice were euthanized or underwent cecal ligation and puncture.

Detection of gfp expression

Mice were anesthetized, and the hind footpad as well as popliteal lymph nodes were harvested and stored in 30% sucrose until frozen sections were made. The tissues were sectioned at 5 μm and then photographed using a fluorescent microscope (Axioskop wide-field fluorescent microscope; Zeiss, New York, NY).

Flow cytometric analysis of lymph node leukocytes

The popliteal lymph node was harvested 24 h after footpad injection. Lymph nodes were first dissected at 4°C with 30-gauge needles, filtered through a 70-μm pore size cell strainer (Falcon, BD Biosciences), then resuspended in 400 U/ml collagenase D (Roche, Norwich, CT) and incubated for 30 min in a 37°C water bath (17). After washing twice with buffer (1% BSA and 1 mM EDTA (Fisher Scientific, Pittsburgh, PA) and 0.1% sodium azide (NaN$_3$; Sigma-Aldrich, St. Louis, MO) in HBSS without phenol red, calcium, and magnesium (Cellgro; Mediatech, Herndon, VA), 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), followed by staining. DCs were characterized using anti-CD11c and anti-CD8 (clone 53-6.7) Abs (Immunotech, Miami, FL). Maturation of DCs was assessed by the levels of MHC class II and CD86 expression. T cells were identified with anti-CD3, anti-CD4, or anti-CD8 Abs. T cell activation was assessed by the expression of CD69 and CD25. Directly conjugated Abs were used for FITC, PerCP, and PE. Samples were acquired and analyzed on a FACSCalibur instrument (BD Biosciences, San Jose, CA).

DNA isolation and PCR

DNA was isolated from popliteal lymph nodes or footpad tissue using the Qiagen DNeasy Tissue Kit (Qiagen, Valencia, CA). PCR was performed with reagents from the GeneAmp PCR Core Kit (PE Applied Biosystems, Foster City, CA). For each sample, 1 μg DNA was added to a 50-μl PCR containing 2 mM MgCl$_2$, 1× PCR buffer II, 0.2 mM dNTPs, 1.25 U AmpliTaq DNA polymerase, and 50 μmol of specific oligonucleotide primers. The primer sequences for hIL-10 were 5′-CGGCCGCTC GAGTCTAGAC-3′ and 5′-GTGGATAGCGGTTTGACTCAC-3′. The PCR temperature profile consisted of a single cycle at 94°C for 2 min; 35 cycles for 1 min at 94°C (denaturing), 1 min at 60°C (annealing), and 2 min at 72°C (extension); and a final cycle at 72°C for 7 min. The PCR products were electrophoresed on a 2% agarose gel containing 0.4 μg/ml ethidium bromide.

Cecal ligation and puncture model of polymicrobial sepsis

For induction of a polymicrobial sepsis, mice were subjected to cecal ligation and puncture as previously described (18). In brief, laparotomy was performed, and the cecum was isolated, ligated, and punctured through and through with a 22-gauge needle. Depending on the experiment, mice were either euthanized at 24 h after surgery, and organs and blood were harvested, or animals were observed for up to 10 days to determine outcome.

Caspase-3 activity assay

Protein extracts were prepared by homogenization of thymus tissues, and caspase-3 activity was determined by a fluorometric assay (Enzyme Systems Products, Livermore, CA). Harvested thymi were homogenized in 1 ml 25 mM HEPES buffer (pH 7.5) containing 5 mM MgCl$_2$, 1 mM EGTA, 1 mM PMSF, 1 μg/ml leupeptin, and aprotinin. After centrifugation at 13,500 rpm for 15 min, the supernatants were collected. Protein concentrations in the supernatant were assayed using a protein assay kit (Bio-Rad, Hercules, CA). Fifty micrograms of the extracted proteins were incubated with the synthetic fluorescent substrate benzylooxycarbonyl-Asp-Glu-Val-Asp-7-amino-4-trifluoromethylcoumarin at a concentration of 20 mM in 0.1 M HEPES buffer (pH 7.4) containing 2 mM DTT, 0.1% 3-[93-chol-amidopropyl]dimethylammonio]-1-propane-sulfonate, and 10% sucrose. The kinetics of the proteolytic cleavage of the substrates were monitored in a fluorescence microplate reader using an excitation wavelength of 360 nm and an emission wavelength of 535 nm. The fluorescence intensity was calibrated with a standard concentration of 7-amino-4-trifluoromethylcoumarin, and the caspase activity was calculated from the slope of the recorded fluorescence and expressed as the relative fluorescence intensity.

Cytokine measurements

Murine IL-6 and murine and hIL-10 in plasma were measured by specific ELISA using commercially available reagents. A minikit for murine IL-6 (Endogen, Cambridge, MA) was used, whereas the human and murine IL-10 ELISAs used Ab pairs (hIL-10, clones 9D7 and 12G8 (R&D Systems, Minneapolis, MN); mIL-10, JES052A5 (Endogen)).

Presentation of data and statistics

Results are presented as the mean ± SEM. Differences between experimental groups were considered significant at $p < 0.05$, as determined by one-way ANOVA. Post-hoc analyses were performed with Student-Newman-Keuls multiple range test. Differences in survival were determined with Kaplan-Meier log-transformed survival analysis.

Results

Adv infects DCs in vivo

Particles (2×10^5 or 2×10^{10}) of recombinant Adv expressing gfp or containing an empty cassette were injected into both hind footpads of mice. Twenty-four hours later, the draining popliteal

lymph nodes were harvested and examined for gfp by either frozen section or flow cytometry. Frozen sections of the popliteal lymph nodes showed fluorescent cells distributed in the subcapsular and T cell areas of the lymph node (Fig. 1a). Analysis of the lymph nodes by flow cytometry revealed that ~27% of CD11c$^+$, MHC class II-expressing cells were gfp positive, indicating that DCs had been transduced with the recombinant Adv and were expressing the transgene (Fig. 1b). Additional flow analysis revealed that there were essentially no gfp-positive cells in the CD3$^+$, CD4$^+$, and CD8$^+$ cell populations, confirming that T cells are not readily transduced by Adv (data not shown). Examining the DC population in greater detail revealed that lymphoid DCs (CD8$^+$CD11c$^+$MHC class II^{high}CD86^{high}) were transduced to a greater degree than were myeloid DCs (CD8$^-$CD11c$^+$MHC class II^{high}CD86^{high}). Three percent of all lymphoid DCs in the lymph node were gfp positive following injection of 2×10^5 particles of recombinant Adv, whereas nearly 30% were gfp positive following injection of 2×10^{10} particles of the same vector. In contrast, 0.5 and 10% of myeloid DCs were also gfp positive following administration of 2×10^5 and 2×10^{10} particles, respectively (data not shown).
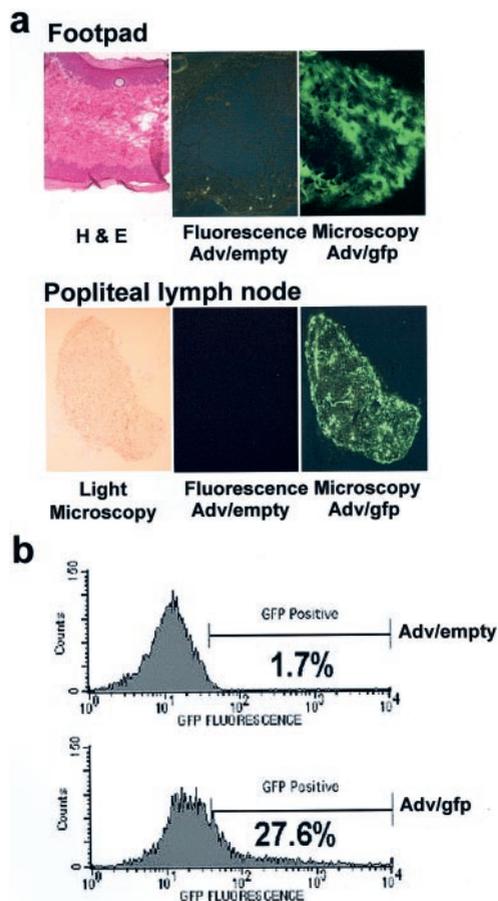

FIGURE 1. Detection of gfp-positive cells in the footpad and popliteal lymph node (one representative figure from four repetitions). *a*, Fluorescent microscopy of frozen section of footpad as well as popliteal lymph node were examined 24 h following footpad injection of 2×10^{10} particles of an adenoviral vector either expressing gfp (Adv/gfp) or containing an empty cassette (Adv/empty). *b*, Flow cytometric analysis was performed on the popliteal lymph node and gated on live CD11c$^+$ and high MHC class II cells, revealing that 27% of these cells had been transduced with the adenoviral vector expressing gfp.

Adv expressing hIL-10 decreases DC maturation and lymphocyte activation

Because DCs are activated by Adv in vitro (14), we examined the status of DC maturation in CD8$^+$ and CD8$^-$ subsets 24 h after footpad administration of a recombinant Adv containing an empty cassette (Adv/empty). We also administered Adv expressing hIL-10 (Adv/hIL-10) to determine whether the expression of this anti-inflammatory cytokine blocked DC maturation as reported with administration of the recombinant protein (10). In addition, because DCs can stimulate T cell activation, we determined whether hIL-10 expression in DC within the popliteal lymph node would regulate CD4$^+$ or CD8$^+$ lymphocyte activation by assessing CD69 or CD25 expression following viral exposure. Treatment with 2×10^{10} particles of the Adv containing an empty cassette led to maturation of both lymphoid and myeloid DCs in the popliteal lymph node, as demonstrated by up-regulation of CD86 surface Ag (Fig. 2a). Interestingly, treatment of mice with the recombinant vector expressing hIL-10 at the same dose (10^{10} particles) reversed this effect, with CD86 expression below the levels seen in DCs from mice injected with buffer alone.

Injection of 2×10^5 particles of the adenoviral vector with an empty cassette led to only a minimal activation of CD4$^+$ and CD8$^+$ T cells (Fig. 2b). Approximately 2% more CD4$^+$ and CD8$^+$ cells expressed CD69 after adenoviral injection compared with those after buffer treatment. Injection of a higher particle dose (2×10^{10}) led to a nearly 6-fold increase in the number of activated CD4$^+$ and CD8$^+$ T cells. Interestingly, administration of 2×10^{10} particles of the vector expressing hIL-10 (Adv/hIL-10) reversed the effect, leading to a similar degree of activation as that seen with the lower particle dose (Fig. 2b).

Local vs systemic appearance of IL-10 is dose dependent

One possible advantage of using IL-10 therapies for the treatment of inflammatory disease is the capacity to achieve only local expression, as systemic exposure to this cytokine often leads to unwanted immunosuppressive effects (19). As demonstrated by Steinhauser et al. (20), systemic administration of IL-10 suppressed the immune response to *Pseudomonas* pneumonia following cecal ligation and puncture. Therefore, one of the goals of these studies was to establish whether local adenoviral expression of IL-10 could be obtained. Therefore, we administered different quantities of Adv expressing hIL-10 into the footpad of healthy mice to determine whether local production in the draining lymph nodes, in the absence of systemic appearance in the circulation, could be achieved. At 24 h we analyzed plasma for hIL-10, and lymph nodes for hIL-10 cDNA. With a dose of 2×10^5 particles, we could not detect hIL-10 in the plasma of recipient animals (Fig. 3a). In marked contrast, higher doses of Adv expressing IL-10 (2×10^7 particles and 2×10^{10} particles, respectively) showed a dose-dependent increase in the plasma appearance of hIL-10.

To confirm that the adenoviral delivery of IL-10 was indeed localized to the draining lymph nodes, we examined hIL-10 cDNA in the popliteal lymph node and at the site of injection (footpad), as well as at distant sites, such as the inguinal lymph node, spleen, thymus, lung, and liver. hIL-10 cDNA could be detected in the popliteal lymph nodes as well as in the injected footpad on postinjection days 1 and 3 (Fig. 3b). In contrast, in only one of three replicates could hIL-10 cDNA be recovered from the more distant inguinal lymph node. No hIL-10 cDNA could be recovered from any other organ, as determined by PCR (data not shown).
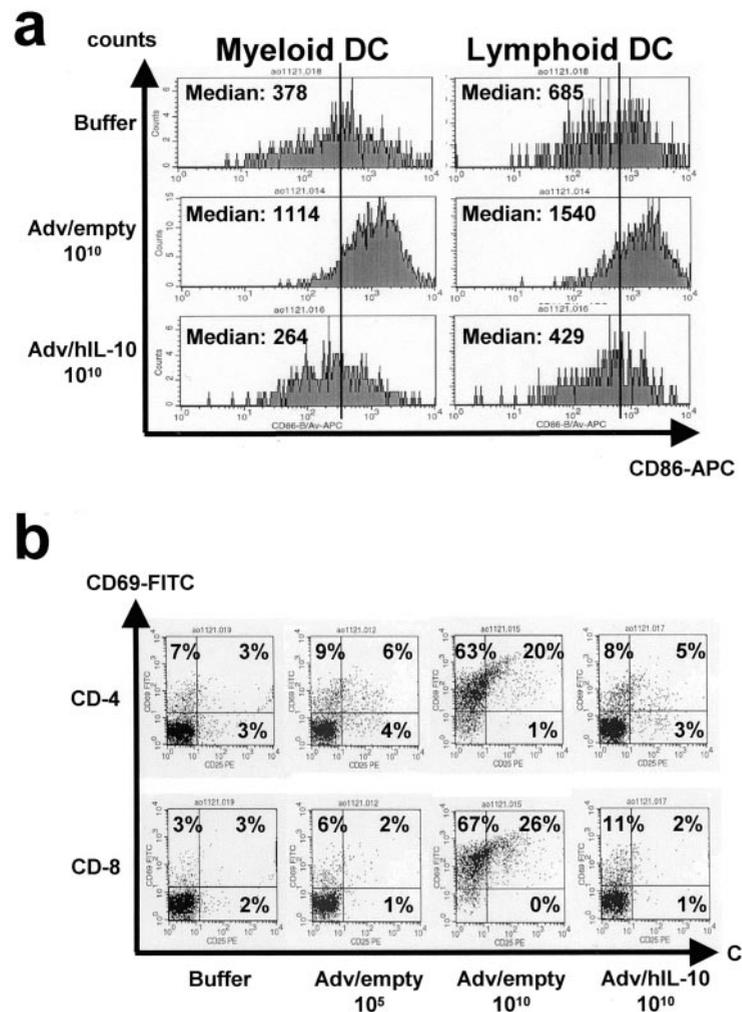
FIGURE 2. Adv-induced DC and T cell activation. Flow cytometric analysis of popliteal lymph node (one representative analysis from four repetitions) 24 h after footpad injection of either 2×10^5 or 2×10^{10} particles of Adv expressing hIL-10 (Adv/hIL-10) or containing an empty cassette (Adv/empty). *a*, DC were stained and gated for either the myeloid population as CD11c$^+$-PE, CD8-PerCP$^-$ and class II-FITC^{high} or the lymphoid population as CD11c$^+$-PE, CD8-PerCP$^+$, and class II-FITC^{high}, and activation was measured by the up-regulation of CD86$^+$-allophycocyanin. *b*, T cells were stained for CD4$^+$-allophycocyanin and CD8$^+$-PerCP, and their activation was determined by up-regulation of the early activation marker CD69-FITC and the late activation marker CD25-PE.

Local expression of hIL-10 leads to increased survival of septic mice

Having demonstrated that local expression of IL-10 could be achieved, we next determined whether the compartmentalized expression of this cytokine in peripheral lymph nodes would affect the survival of mice developing polymicrobial sepsis following cecal ligation and puncture. To obtain local expression of IL-10, mice were pretreated with footpad injections of 2×10^5 particles of Adv expressing hIL-10 or containing an empty cassette, respectively, 24 h before cecal ligation and puncture. Mice pretreated with the recombinant expressing hIL-10 had a significant increase in survival (55%, 11 of 20), whereas animals receiving the empty vector control (Adv/empty) died at the same rate as mice injected with buffer (both 25%, 5 of 20; Fig. 4). Furthermore, we tested the effect on sepsis in mice treated with the high particle dose of 2×10^{10} of the respective Adv vectors (Adv/hIL-10, Adv/empty, and buffer control only), a dose that resulted in systemic exposure of hIL-10 (see Fig. 3). In contrast to the former study, there was no difference in survival among the treatment groups (Adv/empty, 4 of 20; Adv/hIL-10, 5 of 20; buffer control, 4 of 20).

Plasma IL-6 and murine IL-10 levels and thymic caspase-3 activity are not associated with improved outcome

Twenty-four hours following cecal ligation and puncture, and thus 48 h after footpad injection of 2×10^5 particles, plasma IL-6 and murine IL-10 levels as well as thymic caspase-3 activity were determined. Earlier studies had shown that increased levels of these cytokines and increased thymic apoptosis correlate with an adverse outcome in similar animal models and in patients with sepsis (21, 22). At this time point, however, no differences among the three treatment groups were seen despite significant differences in outcome (Fig. 5). There was, however, a trend toward reduced plasma IL-6 and increased murine IL-10 levels as well as decreased thymic caspase-3 activity in the group of mice treated with footpad injection of Adv expressing hIL-10 (Fig. 5).

Discussion

In the present report we demonstrate that following s.c. injection, recombinant adenoviral vectors naturally target DCs in the draining lymph nodes, and this observation correlates well with in vitro observations (our unpublished observations) (14). We found that within 24 h footpad injection with recombinant Adv targets both myeloid and lymphoid DCs in the draining popliteal lymph node and results in protein expression. The appearance of transduced popliteal lymph node DCs could be explained by lymphatic trafficking of Adv from the footpad to the lymph node, where both lymphoid-related and myeloid-related DCs are directly transduced. Alternatively, myeloid DCs and other APC residing in the interstitial tissue of the footpad may be transduced directly and then migrate to the draining lymph node. If so, then Adv transduction of CD8$^+$ DCs may occur as a result of phagocytosis of myeloid DCs by these cells (23). Regardless of how each DC population is transduced, these findings are of significant potential interest, as DCs
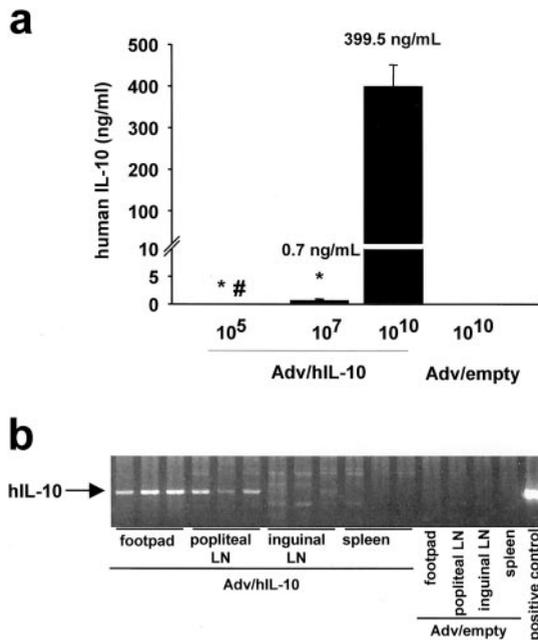
FIGURE 3. Plasma hIL-10 concentrations and expression in the lymph node. *a*, hIL-10 was measured in the plasma 24 h after injection in each hind footpad of 2×10^5, 2×10^7, or 2×10^{10} particles of Adv/hIL-10 or Adv/empty ($n = 5$). *b*, PCR analysis for hIL-10 cDNA was performed on the popliteal and inguinal lymph nodes, spleen, and footpad 24 h after footpad injection of 2×10^5 particles of either Adv/hIL-10 or Adv/empty. *, $p < 0.05$, 10^5 and 10^7 vs 10^{10}; #, $p < 0.05$, 10^5 vs 10^7.

can be targeted in vivo in a lymph node compartment for the expression of genes that may regulate their function and immune responses.

This study also demonstrates a dose effect of Adv on in vivo DC activation, such that higher particle numbers of Adv (10^{10}) promote DC maturation, while lower numbers (10^5) do not cause significant DC maturation in vivo. We also found a similar dose effect for activation in in vitro-derived DC cultures (data not shown). Although only 30% of all DCs in the lymph node are transduced following administration of 10^{10} particles, the majority of both CD8^{+} and CD8^{−} DC populations in the popliteal lymph node are mature at 24 h following injection. In addition, popliteal lymph node T cells are activated with this dose of Adv. This suggests that at higher doses recombinant Adv may act as an adjuvant of trans-
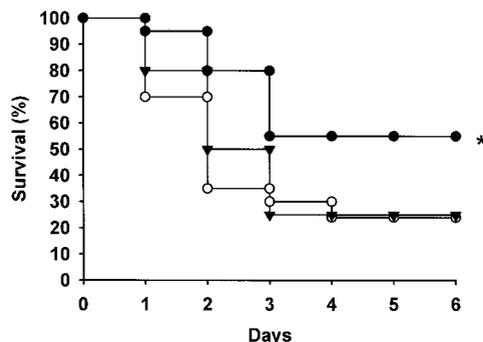

FIGURE 4. Survival rate of septic mice pretreated with Adv. A survival study ($n = 20$ for each group) was performed by pretreating mice with footpad injections in both footpads of 2×10^5 particles of Adv/hIL-10 (●), Adv/empty (○), or buffer (▼). Twenty-four hours later, mice underwent cecal ligation and puncture and were observed for 6 days. *, $p < 0.05$, Adv/hIL-10 vs Adv/empty or buffer.
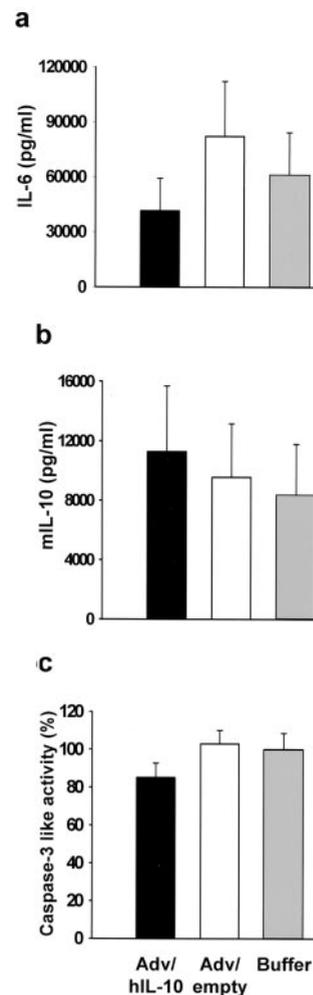

FIGURE 5. Cytokine response and thymic apoptosis. Pretreated mice ($n = 5$) injected in both hind footpads with 2×10^5 particles of Adv/hIL-10, Adv/empty, or buffer underwent cecal ligation and puncture 24 h later. After an additional 24 h, mice were euthanized. IL-6 (*a*) and mIL-10 (*b*) were measured in plasma and thymic caspase-3 activity was determined (*c*).

duced as well as uninfected DCs and subsequent T cell activation. DC maturation may be induced directly by viral DNA or proteins derived from the virions (2). Alternatively, maturation may result from the paracrine actions of IFN-γ produced as a consequence of transduction-mediated induction of inflammatory cytokines produced by infected DCs, macrophages, or activated T cells (2). Importantly, DC maturation as well as T cell activation are efficiently blocked in vivo when recombinant Adv expresses hIL-10. Previous in vitro studies suggest that exposure of immature DCs to IL-10 blocks maturation and makes them resistant to maturation stimuli applied at later time points (24). Although we have not determined that DCs expressing the IL-10 transgene in vivo are resistant to subsequent maturation stimuli, we have found that in vitro transduction of DCs with Adv expressing IL-10 will still undergo phenotypic maturation with LPS. However, the production of IL-12 by Adv/hIL-10 transduced cells in response to LPS-induced maturation remains suppressed (our unpublished observations). These results, when considered in the context of the findings of Steinbrink et al. (24), suggest that the adenoviral vector itself may modify the DC response to IL-10, and IL-10 may modify the DC response to adenoviral vector transduction in vivo.

Perhaps one of the most interesting findings is that targeted expression of IL-10 in popliteal lymph node DCs provides significant

protection from the lethal effects of generalized peritonitis. Based upon our studies reported here, only 3% of the DCs in the draining lymph node were transduced following injection with 10^5 particles of Adv, and IL-10 expression was localized to the popliteal lymph node and footpad. Expression of hIL-10 cDNA was not reproducibly observed in any other lymphoid structure or organ, and IL-10 protein was not detected in the circulation.

A central question is why does compartmentalized local expression of IL-10 improve the outcome in sepsis, while systemic production does not. Previous studies examining the role of systemic endogenous IL-10 in the pathogenesis of sepsis are somewhat confounding, but some suggest that systemic expression of this cytokine contributes to untoward immune suppression and mortality following sepsis (20, 25). Other studies with systemic administration of exogenous IL-10 have also demonstrated increased mortality (26). In a recent report we demonstrated that i.v. administration of recombinant Adv expressing IL-10 failed to protect mice from mortality in an identical cecal ligation and puncture model of sepsis. In contrast, when the same vector was administered intrathymically, survival was significantly improved (27, 28). In these same studies intrathymic injection of Adv led to local expression, primarily in DCs, whereas the nonprotective i.v. administration of Adv resulted in hepatocyte transduction and the systemic appearance of IL-10. We are, therefore, led to postulate that local contained expression of IL-10 in certain primary and secondary lymphoid organs may promote host survival during the sepsis response, in contrast to the systemic expression of this cytokine. This hypothesis is consistent with studies where high systemic levels of endogenous IL-10 predict adverse outcome in septic and trauma patients (29), presumably by causing generalized immune suppression and a predominant Th2 response, as seen after thermal injuries and generalized peritonitis (30).

Beyond the issue of local expression, the capacity to target DCs and the ability of IL-10 to maintain an immature DC phenotype in a lymphoid structure may be of critical importance in regulating the sepsis response. Previous studies suggest that DCs are lost from tissues during sepsis (31), perhaps due to activation-induced cell death compromising the acquired immune responses to pathogens. Our in vitro studies suggest that IL-10 expression in Adv/hIL-10 transduced DCs does not suppress increases in CD86 and MHC class II Ags following LPS stimulation, but does limit IL-12 production (our unpublished observations). Therefore, the presence of immature or mature DC without the capacity to produce normal amounts of IL-12 could promote the generation of regulatory T cells (32) that may limit untoward aspects of the acquired or innate immune responses in the setting of sepsis. We are currently examining these possibilities.

As we did not assess the cell phenotype from the footpad of Adv-injected mice, these studies may underestimate the total number of DCs transduced. If tissue myeloid DCs in the footpad were transduced at the site of injection, IL-10 expression may have interfered with the maturation of these cells, limiting their expression of chemokine receptors, and their ability to migrate to the draining lymph node. In fact, Takayama et al. (33) demonstrated that exposure of immature DCs to IL-10 prevents their expression of CCR7 and impairs the homing of these cells to secondary lymphoid tissues. Furthermore, IL-10 exposure of DCs results in their expression of chemokine decoy receptors that fail to signal and elicit migration and serve only as a sink for locally produced inflammatory chemokines (34).

Overall, these studies provide a new in vivo approach to modify murine DC function that allows direct manipulation of the immune response. Our data also demonstrate that gene targeting of DCs with IL-10 may be useful for acute infectious diseases, such as

sepsis syndromes. Although the mechanism of action is not resolved, compartmentalized expression of IL-10 in DCs clearly alters the response to sepsis. Further studies will be required to more fully delineate the cellular mechanisms by which compartmentalized modification of DC function impacts host immunity and outcome in sepsis.

References

1. Steinman, R. M. 1991. The dendritic cell system and its role in immunogenicity. *Annu. Rev. Immunol. 9:271.*
2. Pulendran, B., K. Palucka, and J. Banchereau. 2001. Sensing pathogens and tuning immune responses. *Science 293:253.*
3. Reis e Sousa, C. 2001. Dendritic cells as sensors of infection. *Immunity 14:495.*
4. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature 392:245.*
5. 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.*
6. Oberholzer, A., C. Oberholzer, R. M. Minter, and L. L. Moldawer. 2001. Considering immunomodulatory therapies in the septic patient: should apoptosis be a potential therapeutic target? *Immunol. Lett. 75:221.*
7. Karp, C. L., M. Wysocka, X. Ma, M. Marovich, R. E. Factor, T. Nutman, M. Armant, L. Wahl, P. Cuomo, and G. Trinchieri. 1998. Potent suppression of IL-12 production from monocytes and dendritic cells during endotoxin tolerance. *Eur. J. Immunol. 28:3128.*
8. Wysocka, M., S. Robertson, H. Riemann, J. Caamano, C. Hunter, A. Mackiewicz, L. J. Montaner, G. Trinchieri, and C. L. Karp. 2001. IL-12 suppression during experimental endotoxin tolerance: dendritic cell loss and macrophage hyporesponsiveness. *J. Immunol. 166:7504.*
9. Faulkner, L., G. Buchan, and M. Baird. 2000. Interleukin-10 does not affect phagocytosis of particulate antigen by bone marrow-derived dendritic cells but does impair antigen presentation. *Immunology 99:523.*
10. De Smedt, T., M. M. Van, G. De Becker, J. Urbain, O. Leo, and M. Moser. 1997. Effect of interleukin-10 on dendritic cell maturation and function. *Eur. J. Immunol. 27:1229.*
11. Corinti, S., C. Albanesi, A. la Sala, S. Pastore, and G. Girolomoni. 2001. Regulatory activity of autocrine IL-10 on dendritic cell functions. *J. Immunol. 166:4312.*
12. Rea, D., F. H. Schagen, R. C. Hoeben, M. Mehtali, M. J. Havenga, R. E. Toes, C. J. Melief, and R. Offringa. 1999. Adenoviruses activate human dendritic cells without polarization toward a T-helper type 1-inducing subset. *J. Virol. 73:10245.*
13. Zhong, L., A. Granelli-Piperno, Y. Choi, and R. M. Steinman. 1999. Recombinant adenovirus is an efficient and non-perturbing genetic vector for human dendritic cells. *Eur. J. Immunol. 29:964.*
14. Morelli, A. E., A. T. Larregina, R. W. Ganster, A. F. Zahorchak, J. M. Plowey, T. Takayama, A. J. Logar, P. D. Robbins, L. D. Falo, and A. W. Thomson. 2000. Recombinant adenovirus induces maturation of dendritic cells via an NF-κB-dependent pathway. *J. Virol. 74:9617.*
15. Graham, F. L., and L. Prevec. 1995. Methods for construction of adenovirus vectors. *Mol. Biotechnol. 3:207.*
16. Minter, R. M., M. A. Ferry, J. E. Rectenwald, F. R. Bahjat, A. Oberholzer, C. Oberholzer, D. Laface, V. Tsai, I. Amed, E. M. Copeland, et al. 2001. Extended lung expression and increased tissue localization of viral IL-10 with adenoviral gene therapy. *Proc. Natl. Acad. Sci. USA 98:277.*
17. Salomon, B., J. L. Cohen, C. Masurier, and D. Klatzmann. 1998. Three populations of mouse lymph node dendritic cells with different origins and dynamics. *J. Immunol. 160:708.*
18. 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.*
19. Moore, K. W., M. R. de Waal, R. L. Coffman, and A. O'Garra. 2001. Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol. 19:683.*
20. Steinhauser, M. L., C. M. Hogaboam, S. L. Kunkel, N. W. Lukacs, R. M. Strieter, and T. J. Standiford. 1999. IL-10 is a major mediator of sepsis-induced impairment in lung antibacterial host defense. *J. Immunol. 162:392.*
21. Damas, P., A. Reuter, P. Gysen, J. Demonty, M. Lamy, and P. Franchimont. 1989. Tumor necrosis factor and interleukin-1 serum levels during severe sepsis in humans. *Crit. Care Med. 17:975.*
22. 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.*
23. Inaba, K., S. Turley, F. Yamaide, T. Iyoda, K. Mahnke, M. Inaba, M. Pack, M. Subklewe, B. Sauter, D. Sheff, et al. 1998. Efficient presentation of phagocytosed cellular fragments on the major histocompatibility complex class II products of dendritic cells. *J. Exp. Med. 188:2163.*
24. Steinbrink, K., M. Wölfl, H. Jonuleit, J. Knop, and A. H. Enk. 1997. Induction of tolerance by IL-10-treated dendritic cells. *J. Immunol. 159:4772.*
25. Remick, D. G., S. J. Garg, D. E. Newcomb, G. Wollenberg, T. K. Huie, and G. L. Bolgos. 1998. Exogenous interleukin-10 fails to decrease the mortality or morbidity of sepsis. *Crit. Care Med. 26:895.*
26. van der Poll, T., A. Marchant, C. V. Keogh, M. Goldman, and S. F. Lowry. 1996. Interleukin-10 impairs host defense in murine pneumococcal pneumonia. *J. Infect. Dis. 174:994.*
27. Minter, R. M., M. A. Ferry, M. E. Murday, C. L. Tannahill, F. R. Bahjat, C. Oberholzer, A. Oberholzer, D. Laface, B. Hutchins, S. Wen, et al. 2001.

Adenoviral delivery of human and viral il-10 in murine sepsis. *J. Immunol. 167: 1053.*

28. Oberholzer, C., A. Oberholzer, F. R. Bahjat, R. M. Minter, C. Tannahill, A. Abouhamze, D. Laface, B. Hutchins, M. 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.*

29. Neidhardt, R., M. Keel, U. Steckholzer, A. Safret, U. Ungethuem, O. Trentz, and W. Ertel. 1997. Relationship of interleukin-10 plasma levels to severity of injury and clinical outcome in injured patients. *J. Trauma 42:863.*

30. Lyons, A., J. L. Kelly, M. L. Rodrick, J. A. Mannick, and J. A. Lederer. 1997. Major injury induces increased production of interleukin-10 by cells of the immune system with a negative impact on resistance to infection. *Ann. Surg. 226:450.*

31. Hotchkiss, R. S., K. W. Tinsley, P. E. Swanson, R. E. J. Schmieg, J. J. Hui, K. C. Chang, D. F. Osborne, B. D. Freeman, J. P. Cobb, T. G. Buchman, et al.

2001. Sepsis-induced apoptosis causes progressive profound depletion of B and CD4$^+$ T lymphocytes in humans. *J. Immunol. 166:6952.*

32. Yang, J. S., L. Y. Xu, Y. M. Huang, P. H. Van der Meide, H. Link, and B. G. Xiao. 2000. Adherent dendritic cells expressing high levels of interleukin-10 and low levels of interleukin-12 induce antigen-specific tolerance to experimental autoimmune encephalomyelitis. *Immunology 101:397.*

33. Takayama, T., A. E. Morelli, N. Onai, M. Hirao, K. Matsushima, H. Tahara, and A. W. Thomson. 2001. Mammalian and viral IL-10 enhance C-C chemokine receptor 5 but down-regulate C-C chemokine receptor 7 expression by myeloid dendritic cells: impact on chemotactic responses and in vivo homing ability. *J. Immunol. 166:7136.*

34. D'amico, G., G. Frascaroli, G. Bianchi, P. Transidico, A. Doni, A. Vecchi, S. Sozzani, P. Allavena, and A. Mantovani. 2000. Uncoupling of inflammatory chemokine receptors by IL-10: generation of functional decoys. *Nat. Immunol. 1:387.*