Dendritic Cell (DC)-Based Anti-Infective Strategies: DCs Engineered to Secrete IL-12 Are a Potent Vaccine in a Murine Model of an Intracellular Infection

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Dendritic Cell (DC)-Based Anti-Infective Strategies: DCs Engineered to Secrete IL-12 Are a Potent Vaccine in a Murine Model of an Intracelluar Infection

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Infections with intracellular pathogens such as Leishmania donovani and Mycobacterium tuberculosis pose serious health problems worldwide. Effective vaccines for these pathogens are not available. Furthermore, despite optimal therapy, disease progression is often seen with several intracellular infections. For these reasons, we initiated studies to develop novel anti-infective vaccine and treatment strategies that couple the potent Ag-presenting capacity of dendritic cells (DC) with paracrine delivery of potent anti-infective cytokines such as IL-12 to local immune response sites. We tested this strategy in a murine model of visceral leishmaniasis. Adoptive transfer of DCs pulsed ex vivo with soluble L. donovani Ags (SLDA) to naive mice induced the Ag-specific production of IFN-γ, and increased the percentage of activation markers on spleen lymphocytes. SLDA-pulsed DCs engineered by retroviral gene transfer techniques to secrete high levels of biologically active murine IL-12 augmented this immune response further. In several different vaccination and immunotherapy protocols, compared with sham-treated mice, animals receiving SLDA-pulsed DCs either before or following infection had 1–3 log lower parasite burdens, and this protection was associated with a pronounced enhancement in the parasite-specific IFN-γ response. The augmentation of this protection by IL-12-engineered DCs was striking. First, live parasites were not detected in the liver of mice vaccinated with IL-12-transduced, SLDA-pulsed DCs. Second, this parasitological response was associated with a nearly normal liver histology. In contrast, parasites and granulomas were found in mice vaccinated with SLDA-pulsed, nontransduced DCs. Collectively, these studies provide the rationale for the development of potent DC-based immunotherapies. The Journal of Immunology, 1999, 163: 3890–3897.
of tumor Ag-pulsed DCs. To test our hypotheses, we created a retroviral-based gene transfer system to introduce IL-12 into DCs. As a model system of infection by an intracellular organism, we chose murine infection with *Leishmania donovani*, the causative agent for visceral leishmaniasis. This murine model mimics infection by *L. donovani* in humans and control of infection is known to be dependent, in part, on Th1 cell responses (37, 38). To date, effective vaccine Ags for *L. donovani* have not been identified.

Pulsing DCs with live organisms is not an optimal choice when testing a potential vaccine or therapy. We therefore chose a mixture of unfractonated *L. donovani*-derived proteins (soluble *L. donovani* Ag (SLDA)) as a source of microbial Ags to load DCs (39). In this system, we demonstrate first that adoptive transfer of microbial Ag-pulsed DCs induces an Ag-specific Th1 response in vivo. Second, microbial Ag-pulsed DCs administered either before or following infection with *L. donovani* were effective in reducing the parasite burden. Third, physiologically relevant anti-inflammatory immune responses initiated by microbial Ag-pulsed DCs can be amplified by paracrine delivery of IL-12. Finally, the anti-inflammatory potency of microbial Ag-pulsed DCs that have been engineered to secrete high levels of IL-12 is significantly greater than that of nonengineered DCs.

**Materials and Methods**

**Parasites, Ags, and mice**

*L. donovani* 1S strain (MHOM/SD/001S-2D) promastigotes were cultured, and SLDAs were prepared as described previously (38). Stationary phase *L. donovani* promastigotes were inactivated by heating at 55°C for 60 min, and DCs were pulsed with heat-inactivated *L. donovani* at a multiplicity of infection of 10:1. Total RNA was isolated from the stationary phase *L. donovani* promastigotes using the RNeasy kit (Invitrogen, San Diego, CA) according to manufacturer’s instructions. For pulsing DCs with *L. donovani*-derived RNA, the DCs (2.5 × 10⁶ cells/ml) were cultured in 250 μl of serum free Opti-MEM medium (Life Technologies, Gaithersburg, MD) containing 25 μg of RNA and 50 μg of [1-(2,3-dioleoxyloxy)propyl] N,N,N-trimethyl ammonium methylsulfate (DOTAP, Boehringer, Mannheim, Indianapolis, IN) (32). nu/nu (BALB/c) background mice were obtained from the Veterinary Medical Unit breeding colony, Audie Murphy Veterans Affairs Hospital (San Antonio, TX). The phenotype of nu/nu BALB/c mice is identical to that of BALB/c mice. Six-week-old mice were used for all studies. Mice were infected by i.v. (tail vein) injection of 0.5–1 × 10⁷ stationary phase promastigotes.

**Enrichment of DCs**

Bone-marrow derived DCs were generated according to published methods (40). The femurs and tibias were flushed with 3–5 ml of PBS in 1% BSA. Particulate matter was filtered and the RBC were lysed. Bone marrow cells were differentiated into DCs by culturing in RPMI (Life Technologies) supplemented with 10% FCS, 10 μg/ml gentamicin, and recombinant murine cytokines (R & D Systems, Minneapolis, MN) GM-CSF (50 ng/ml) and IL-4 (1 ng/ml) for 7–10 days. On days 3 and 5, the nonadherent cells (granulocytes and lymphocytes) were removed and replaced with fresh medium and growth factors. On day 7, the nonadherent cells were removed and plated in either 24- or 96-well plates or TissueTek (Nunc, Naperville, IL) chamber slides for pulsing studies. In five separate experiments, bone marrow-derived DCs were stained and analyzed by FACS using DC, monocyte, and lymphocyte cell surface markers. Phenotypically bone marrow-derived DCs expressed abundant MHC class II, CD80, CD40, CD11b, NLC145, and CD11c (data not shown). In proliferation assays, there was an increase in thymidine incorporation when SLDA-pulsed DCs were cocultured with lymphocytes derived from lymph nodes/spleens of *L. donovani*-infected mice, indicating that the DCs can present Ags efficiently (data not shown). Thus, the phenotypic and functional characteristics of bone marrow-derived DCs were consistent with previously published data (40).

**Flow cytometry**

Flow cytometry was used to define the phenotypic characteristics of DCs differentiating from bone marrow progenitors. All Abs were obtained from PharMingen (San Diego, CA) except for NLC145 (rat anti-mouse IgG) which was from Caltag (Burlingame, CA). Consistent with other studies, DCs were identified by presence of abundant MHC class II (I-A⁵ haplo-type), NLC145, CD40, CD11c, CD11b, and CD86 staining, and the absence of CD3/CD2 (T cell) and B220 (B cell) staining. T lymphocyte activation in the splenocytes was confirmed by double staining for lymphocyte subsets CD4 or CD8 with CTLA-4, CD40 ligand, or CD28.

**Transduction of DCs and retroviral supernatant generation**

Using standard overlap PCR techniques, the two murine IL-12 subunits (cDNAs for p35 and p40) were a kind gift of Dr. U. Gubler, Hoffmann-LaRoche, Nutley, NJ) were linked together by an amino acid polypeptide linker and amplified as a single ampiclon (41). This fusion construct was sequenced on both strands and then cloned into the replication-incompetent retroviral vector, designated as MFGS. MFGS is derived from Moloney murine leukemia virus (42, 43). The transfection/transduction protocol was as described (42, 43). Briefly, DNA from the MFGS-murine IL-12 (36 μg) and the SV2Neo (4 μg) constructs were transfected into a producer packaging cell line iP cre by calcium phosphate precipitation. The SV2Neo construct contains the neomycin-resistant gene. Single cell colonies that were resistant to neomycin selection were picked. Supernatants from these clones contain the retrovirus and murine IL-12 (p70) and were used to transduce NIH-3T3 cells. Supernatants that conferred the NIH-3T3 cells with the highest murine IL-12 production (>20 ng/ml/24 h as measured by ELISA) after one 6-h transduction were identified as high titer clones. The sandwich ELISA (PharMingen) used measures p70, the bioactive form of murine IL-12. The high titer clone was expanded, and supernatants from this clone were frozen in aliquots of 3 ml. For transduction, the retrovirus supernatants were thawed and DCs were transduced in fresh medium containing 6 μg/ml polybrene (Sigma, St. Louis, MO) and growth factors GM-CSF (50 ng/ml) and IL-4 (1 ng/ml) diluted 1:1 with viral supernatant. DCs were transduced for 6 h on 2 or 3 consecutive days. IL-12 levels were measured in the supernatants from DC cultures 48 h following the last transduction.

**ELISA and in vitro spleen responses**

Splenocytes (5 × 10⁶/ml) were cultured in medium alone or stimulated with 25 μg/ml of SLDA, and supernatants were harvested at 24 and 48 h for analysis of IFN-γ and IL-4 concentrations by a sandwich ELISA (PharMingen). ELISA was used to analyze the serum Ig subtypes (PharMingen). The levels were quantified by comparing the optical density (OD) of the sample to the OD of known standards analyzed simultaneously in the same assay. The cut-off for ELISA assay was 7.5–500 pg/ml for IL-4 and 15.6–500 pg/ml for IFN-γ. To ensure that the OD values fall within the standard curve, the samples for IFN-γ were diluted 1:10 and 1:100.

**Vaccination and immunotherapy studies**

DC preparations were divided into two parts, one of which was pulsed with SLDA (cultured with 25 μg/ml of SLDA for 12 h) and the other was used as an unpulsed control. These two DC preparations were aliquoted and then injected i.v. into mice. In all studies, DC preparations (unpulsed DCs, SLDA-pulsed DCs, IL-12-transduced DCs, or IL-12-transduced, SLDA-pulsed DCs) were washed several times in PBS, and 10⁶ DCs were resuspended in a 100 μl volume for tail vein injection at the time points specified. The Animal Committee of the University of Texas Health Science Center, San Antonio, TX, approved these studies.

**Parasite burden**

At various time points following infection, the parasite burden was quantified in the liver and/or spleen by limiting-dilution culture as described previously (38, 44). Briefly, liver and spleen tissue (20 mg) was homogenized between two sterile frosted end microscopic slides in 2 ml of culture medium (Grace’s insect medium enriched with 15% heat inactivated FBS). The homogenate was resuspended at 1 mg/ml and placed in 96-well flat-bottom plates in 5-fold serial dilutions and cultured for 2 wk. The wells were then scored for presence of parasites, and the reciprocal of the dilution of the final positive well was expressed as log tissue parasite burden per mg of tissue.

**PCR**

Genomic DNA was isolated from the spleen of each mouse, and serial 10-fold dilutions were used for PCR. The PCR primers specific for *Leishmania* species (forward primer, 5′-ATT TTA CAC CAA CCC CAG TT-3′; reverse primer, 5′-ATT TTA CAC CAA CCC CAG TT-3′) amplify a 125-bp DNA fragment (45). The PCR products were analyzed on an ethidium bromide-stained, 2% agarose gel.
Engineering DCs to constitutively produce biologically active IL-12

For paracrine delivery of IL-12, we generated a replication-incompetent retrovirus encoding the two subunits of murine IL-12 as a fusion construct. Following three transductions, the range of IL-12 detected in the supernatants of DC cultures was 6–32 ng/ml (n = 14 transductions). These levels were significantly higher than that detected in the supernatants of nontransduced, nonstimulated murine DCs (10–400 pg/ml; n = 20 transductions). The average daily rate of bioactive IL-12 produced by 10^6 DCs following two or three transductions was 3305 ± 399 pg and 6578 ± 437 pg, respectively. The protocol used to generate DCs from bone marrow cells results in the generation of a mixture of APCs, with the predominant subtype being DCs. Because there is no specific marker for DCs, we did not attempt to quantify the percentage of DCs that was transduced with IL-12. However, considering the high levels of IL-12 detected in the supernatants of transduced DCs, the transduction efficiency of this retroviral gene transfer system is likely to be very high.

Phenotypically, IL-12-transduced DCs and nontransduced DCs expressed similar amounts of CD11b, CD11c, MHC I and II, the costimulatory molecules CD54, CD80, and the DC marker NLDC145 (Fig. 1 and data not shown). However, the expression of CD40 and CD86 was significantly higher in the IL-12-transduced DCs (Fig. 1). Based on this expression pattern, it is likely that the IL-12-transduced DCs contain a mixture of mature (CD86 and MHC class II positive) and immature of DCs. The percentage of macrophages (N418 positive and NLDC145 negative) was similar in the transduced and nontransduced DC groups. Pulsing IL-12-transduced DCs with different forms of Leishmania-derived Ags did not alter their ability to produce high levels of IL-12 (Fig. 2). Even after pulsing with Leishmania-derived Ags (Fig. 2) or following in vitro infection with live L. donovani (data not shown), the amount of IL-12 produced by nontransduced DCs never reached the level produced by IL-12-transduced DCs.

In vivo responses induced by adoptive transfer of microbial Ag-pulsed DCs

We first examined the function of microbial Ag-pulsed DCs after adoptive transfer to naive mice. An Ag-specific IFN-γ response was observed in splenocytes derived from mice that received SLDA-pulsed DCs (Fig. 3, lanes 5 and 6). This response was observed as early as 2 days following adoptive transfer of SLDA-pulsed DCs, peaked at day 14, declined by day 42, and was absent at day 180 (Fig. 3, b–d, compare lanes 5 and 6; and data not shown). Notably, the response at day 42 was greater than that observed at day 2 but less than that at day 14. Unpulsed DCs or PBS did not induce an Ag-specific IFN-γ response (Fig. 3, b–d, lanes 1–4). In an additional control, spontaneous production or an Ag-induced IFN-γ response was not detected in splenocytes derived from mice that received i.v. SLDA alone but no DCs (n = 4 experiments).

Because the prevailing paradigm is that IL-12 is a key factor in the initiation of cell-mediated immunity that links innate immunity with Ag-specific adaptive immune responses, we also determined whether delivery of IL-12 via DCs would enhance these Ag-specific responses. The spontaneous release of IFN-γ from splenocytes derived from mice that received unpulsed but IL-12-transduced DCs was high, and IFN-γ levels did not increase following the ex vivo pulsing of the splenocytes with SLDA (Fig. 3, b–d, lanes 7 and 8). This spontaneous production of IFN-γ was evident at both day 2 and 14 following adoptive transfer of IL-12-transduced DCs but was absent at day 42. High spontaneous IFN-γ production levels were also observed at day 2 and day 14 following adoptive transfer of IL-12-transduced, SLDA-pulsed DCs (Fig. 3, b–c, lanes 9 and 10). However, at day 42 a different pattern was
observed. At this time point there was essentially no spontaneous production of IFN-γ and a clear Ag-specific IFN-γ response was evident (Fig. 3d, lanes 9 and 10). Also, the magnitude of the Ag-specific IFN-γ response following adoptive transfer of IL-12-transduced DCs was greater than that of nontransduced DCs (Fig. 3d, compare lanes 6 and 10).

Fourteen days following adoptive transfer of unpulsed or pulsed DCs, low levels of IL-4 were detected in splenocyte culture supernatants (30–100 pg/ml, no differences among groups; data not shown). IL-4 was not detected in splenocyte culture supernatants of mice sacrificed 2 and 42 days post-DC administration or in supernatants from splenocytes derived from mice that received either PBS or SLDA.

The findings presented demonstrate that microbial Ag-pulsed DCs induced an Ag-specific IFN-γ response and that this response was augmented significantly by DCs that had been transduced in vitro with IL-12 (Fig. 3). IFN-γ is known to induce a switch in Ig isotypes with a preferential production of IgG2a and IgG3. Concordant with this observation, we found that adoptive transfer of IL-12-transduced, SLDA-pulsed DCs resulted in high serum levels of IgG2a. The Ig2a levels were nearly 2-fold higher than those detected in mice receiving SLDA-pulsed DCs or PBS. Adoptive transfer of IL-12-transduced and nontransduced DCs also lead to changes in other Ig subtypes (data not shown). However, these differences were not as striking as those observed in IgG2a following administration of IL-12-transduced DCs.

Compared with mice that received PBS, the activation markers CTLA-4 and CD40 ligand were up-regulated (~200–500%) on both CD4 and CD8 T cell splenocytes derived from mice sacrificed 14 days following adoptive transfer of DCs. However, there were no statistically significant differences among the various DC groups (unpulsed DCs, SLDA-pulsed DCs, IL-12-transduced DCs, and SLDA-pulsed/IL-12-transduced DCs; data not shown). These findings suggest that the adoptive transfer of DCs may lead to the activation of both CD4+ and CD8+ T lymphocytes.

**Microbial Ag-pulsed DCs are effective as both a vaccine and therapy**

We determined next the anti-infective efficacy of SLDA-pulsed DCs in a well-characterized murine model of visceral leishmaniasis (38, 39). The major endpoint for protective efficacy in our study was the level of parasite burden in the liver and/or spleen. Compared with PBS-vaccinated mice, those vaccinated i.v. with SLDA-pulsed DCs had an ~1–3 log reduction in parasite burdens (Table I). In the first vaccination protocol (Table I), the protective efficacy of SLDA-pulsed DCs was also observed at 8 wk postinfection (~1 log reduction in parasite burden; data not shown). A

### Table I. Preventive and curative efficacy of SLDA-pulsed DCs

<table>
<thead>
<tr>
<th>Vaccine Groups</th>
<th>Protocol 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Protocol 2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Treatment studies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver (SEM)</td>
<td>Spleen (SEM)</td>
<td>Liver (SEM)</td>
</tr>
<tr>
<td>PBS</td>
<td>5.0 (0.41)</td>
<td>3.6 (0.34)</td>
<td>5.3 (0.20)</td>
</tr>
<tr>
<td>Unpulsed DC</td>
<td>3.5 (0.70)</td>
<td>2.8</td>
<td>ND</td>
</tr>
<tr>
<td>SLDA-pulsed DCs</td>
<td>2.5 (0.17)*</td>
<td>2.6</td>
<td>2.7 (0.20)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.6 (0.50)*</td>
</tr>
</tbody>
</table>

<sup>a</sup> In protocol 1, mice were vaccinated 2 and 4 wk prior to infection, and parasite burden was determined at 4 and 8 wk postinfection; data shown are 4-wk postinfection parasite burdens.

<sup>b</sup> In protocol 2, mice were vaccinated once, 2 wk prior to infection, and parasite burden was determined 7 wk postinfection. In the treatment study, mice were treated once, 1 wk postinfection, and parasite burden determined 4 wk postinfection.

<sup>*</sup> p < 0.03, and †, p < 0.005 for differences between SLDA-pulsed DCs and PBS control. The differences between the unpulsed DCs and PBS control group were not statistically significant. ND, not done.
variable effect was observed with unpulsed DCs. In some experiments the parasite burden following vaccination with unpulsed DCs was similar to that of the PBS-control vaccinated group. In others, a decline in parasite burden was observed, albeit the decrease was not statistically different from those of the control PBS-vaccinated group (Table I and data not shown). Two vaccinations spaced 2 wk apart with Ag alone (SLDA) or with Ag plus an adjuvant (Corynebacterium parvum) was also ineffective (data not shown). We also determined whether vaccination by the s.c. route was efficacious. Mice vaccinated by the s.c. route and then challenged with parasites i.v. had no demonstrable protection (10 mice in vaccine and nonvaccinated groups).

Spontaneous and Ag-induced IFN-γ production following parasite challenge was significantly higher in mice vaccinated with SLDA-pulsed DCs than in infected, PBS-vaccinated mice (Table II). The Ag-independent production of IL-4 following infectious challenge of PBS-vaccinated mice was abrogated by prior vaccination with SLDA-pulsed DCs (Table II).

In addition to decreasing parasite burdens when given before an infection (i.e., as a vaccine), SLDA-pulsed DCs were also effective in treating an established infection. Compared with sham-treated mice, adoptive transfer of SLDA-pulsed DCs resulted in a 1–2 log lower parasite burden (Table I). In a different treatment protocol, starting 1 wk following infection mice were treated weekly for 3 wk. Four weeks following the last treatment, mice treated with SLDA-pulsed DCs had at least 1 log lower parasite burdens in the liver and spleen (data not shown).

Our in vivo studies in noninfected mice showed that IL-12-transduced SLDA-pulsed DCs enhanced significantly the Ag-specific increases in IFN-γ. We therefore next determined whether the protective effect of Ag (SLDA)-pulsed DCs could be enhanced further by DCs engineered to secrete high levels of IL-12. Parasites could not be detected in the liver of mice that had received either one or two vaccinations with IL-12-transduced, SLDA-pulsed DCs, whereas parasites could be detected in mice vaccinated with SLDA-pulsed DCs or IL-12 transduced DCs (Table III and data not shown). These findings were corroborated by a blinded histopathological analysis of the sections from the liver of mice vaccinated with DCs (see Fig. 5 and Table IV). Compared with the mice that received PBS (Fig. 4A), those vaccinated with SLDA-pulsed DCs had lower numbers of granulomas/cell nests, giant cells, and organisms and the size of the cell nests was also smaller (Fig. 4B). In contrast, IL-12-transduced, SLDA-pulsed DCs had a virtually normal liver histology except for rare granulomas identified after scanning many fields (Fig. 4C).

The limiting-dilution culture assay and the histopathology are sensitive techniques to confirm the presence of infection in murine models of experimental leishmaniasis. By both methods it appeared that vaccination with IL-12-transduced, SLDA-pulsed DCs results in sterile immunity. These striking results prompted us to confirm the administration of L. donovani by a PCR-based strategy. Leishmania species-specific genomic DNA could be identified in the spleen of mice vaccinated with SLDA-pulsed (Fig. 5, lanes 5–8) or IL-12-transduced, SLDA-pulsed DCs (Fig. 5, lanes 9–12). However, even by this highly sensitive technique, mice vaccinated with IL-12-transduced, SLDA-pulsed DCs had at least one log lower amounts of L. donovani genomic DNA than mice vaccinated with SLDA-pulsed DCs. The limitation of this PCR-based assay is that we cannot distinguish whether the genomic DNA detected was from viable or nonviable parasites. However, the data shown strongly suggest that the DNA amplified is likely to be from either nonviable parasites or from a very low level of residual infection.

**Discussion**

In this study, we determined the in vivo efficacy of microbial Ag-pulsed and/or IL-12-transduced DCs in preventing or treating an
intracellular infection, namely a murine model of visceral leishmaniasis. There are three main findings of this study. First, microbial Ag-pulsed DCs can induce a \textit{L. donovani} Ag-specific Th1 response in vivo. Second, this immune response can afford protection both in a vaccination as well as treatment format. Third, adoptive transfer of DCs engineered to secrete high levels of the bioactive form of IL-12 enhanced the physiologically relevant anti-infective immune responses, and in the model tested was associated with potent microbicidal properties. These results contrast with the significantly lower efficacy (\(< 1\) log) of non-DC based vaccination approaches for \textit{L. donovani} (46, 47), and complement and extend significantly the results of recent studies that explored DC-based vaccination strategies for other infectious diseases (22–27).

In recent years, there has been a major thrust toward developing DNA vaccines for infectious diseases (48, 49). However, an alternative, and perhaps more physiologic, strategy could be one based on capitalizing on the biology of DCs. For example, because DCs rapidly home to organs of immune response, such as the liver and spleen (50 –52), the protective responses induced might be generated more rapidly than those induced by DNA-based vaccines. This may be especially advantageous in the setting of an established infection that is progressing despite therapy. The scenario of relentless disease progression despite optimal therapy is seen on occasion in coccidioidomycosis and leishmaniasis. With tuberculosis, the now endemic presence of multiple drug-resistant strains makes the development of alternative therapies an urgent problem. In the context of an epidemic where the rapid induction of protective immune responses is critical, this empiric approach to a difficult vaccine development problem could be an effective initial intervention. Furthermore, in many instances the infectious agent

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**FIGURE 4.** Histopathological changes in the liver of mice vaccinated with SLDA-pulsed DCs or IL-12-transduced, SLDA-pulsed DCs and then challenged with \textit{L. donovani}. A representative hematoxylin and eosin-stained section of the liver from mice vaccinated with PBS (A), SLDA-pulsed DCs (B), or IL-12-transduced, SLDA-pulsed DCs (C) and then challenged with \textit{L. donovani} is shown. Sections are from mice (4 wk postinfection) whose microbiological data are shown in Table III. A, Liver section from a PBS control mouse shows numerous clusters of inflammatory cells (granulomas) within the hepatic parenchyma. The clusters contain a compact mixture of cells and multinucleated giant cells that contain intracellular parasites (arrows; \(< 200\) magnification). The inset shows cells containing parasites at higher magnification (arrow; \(< 400\) magnification). B, Liver section from mice vaccinated with SLDA-pulsed DCs showed fewer granulomas. Two kinds of granulomas were observed: compact (well-formed) or poorly formed (arrows; \(< 200\) magnification). The inset shows a loosely arranged cluster of inflammatory cells and a multinucleated giant cell that contains intracellular parasites (arrow; \(< 400\) magnification). C, Liver section from a mouse vaccinated with IL-12-transduced, SLDA-pulsed DCs shows a single small cellular aggregate adjacent to the central vein (arrow; \(< 200\) magnification). Compared with mice vaccinated with PBS or SLDA-pulsed DCs, the number of cells within the cellular aggregates was significantly less (inset, \(< 400\) magnification).

**FIGURE 5.** Leishmania species-specific genomic DNA in the spleen of mice was significantly lower in mice vaccinated with IL-12-transduced, SLDA-pulsed DCs. Genomic DNA was extracted from spleen of mice whose microbiological data are shown in Table III. The PCR amplification products from 2.5 \(\mu\)g (A) and 0.25 \(\mu\)g (B) of genomic DNA derived from the spleen of a noninfected, nonvaccinated mouse (lane 4), or infected mice vaccinated with either SLDA-pulsed DCs (lanes 5–8) or IL-12-transduced, SLDA-pulsed DCs (lanes 9–12) are shown. Lane 2 represents the positive control, i.e., the amplicon from \textit{L. donovani}-derived DNA. Lanes 1 and 3 represent \textit{Hae}III m.w. markers and a PCR water control, respectively. Size markers are in base pairs. The arrow denotes the 125-bp amplicon that represents \textit{Leishmania}-specific DNA (see Materials and Methods).
responsible for an epidemic or an unresponsive infection is a mutant strain that has a phenotype distinct from conventional laboratory or vaccine strains. In this instance, pulsing DCs with microbial Ags derived from the mutant microbial strain may be especially advantageous because microbe-specific responses would be generated. Study of these protective responses may in turn provide insight into important immunoprotective factors that could be applied to conventional vaccines.

A major limitation of the DNA vaccine approach is that the identity of the microbial Ag(s) must be known. In the case of most intracellular microbes, their genomes are very large, and the task of identifying protective Ags is arduous. Furthermore, it is likely that a broad repertoire of microbial Ags is required to induce a potent protective immune response in vivo. Thus, the administration of DCs pulsed with unfractionated pools of microbial Ags has the advantage of inducing a protective polyclonal T cell response directed against yet-to-be identified Ags.

One of the major concepts in cytokine biology is that their activity is most potent when they are expressed in a paracrine fashion, i.e., at the site of the Ag. Cytokines differ from hormones in that they are often secreted locally and usually provide local paracrine effects rather than systemic effects. For this reason, treating patients using systemically administered cytokines may not be the most appropriate method of optimizing local Ag presentation and effector cell function at the sites where they are required. As DCs occupy such a pivotal position in the initiation of the immune response, it seems logical to target these cells for cytokine delivery. Gene transfer techniques have been used for over a decade to deliver high-level expression of cytokines and other gene products within the tumor microenvironment (53). However, the same techniques are applicable for targeting DCs to provide enhanced expression of the cytokine/chemokine of interest in the environment of Ag presentation within secondary lymphoid tissue. Conceivably, one could develop inducible-repressible promoter systems that may allow gene expression to be induced after the DC has entered secondary lymphoid tissue.

In addition to harnessing their potent Ag-presenting properties, we also targeted DCs for the paracrine delivery of IL-12 with the intent of priming the cellular microenvironment to induce a more potent response. Given the central role of IL-12 in the promotion of Th1-type cellular immunity (8, 9), IL-12 delivered directly within sites of active T-cell induction is an attractive approach in the management of serious intracellular infections. Furthermore, the efficacy of IL-12 to serve as an effective biologic adjuvant in DNA and recombinant vaccine formats reinforces the importance of this Th-1 biasing cytokine (8, 9, 54). The efficacy of IL-12 in augmenting the Ag-specific Th1 responses induced by microbial Ag-pulsed DCs in vivo is in agreement with our previous in vitro studies that showed the potential of IL-12-transduced human DCs in inducing Ag-specific Th1 responses and down-modulating a Th2 response (43).

In this study, unpulsed DCs showed some protection. However, in contrast to vaccination or treatment with SLDA-pulsed DCs, the reduction in parasite burden following vaccination or treatment with unpulsed DCs was not statistically significant and the response was not always reproducible. This effect of unpulsed DCs could be due to the nonspecific induction of IFN-γ as well as the activation and maturation of DCs in vivo. Alternatively, it is conceivable that following adoptive transfer, unpulsed DCs process and present the Ags presented to them in vivo, i.e., the microbial Ags of an infectious challenge. Conceivably, the nonspecific effects of DC administration might become less apparent if the challenge infection were given after a longer interval than 2 wk after vaccination. Ongoing experiments are addressing this issue as well as the durability of the protection. Nonspecific effects of DCs have been observed in other systems also. Shimizu et al. (55) recently showed that immunization with DCs break the cytotoxic T lymphocyte tolerance in hepatitis B virus transgenic mice, and was found to be more efficient than DNA immunization in this setting.

In preliminary experiments, we found that the parasite burden in animals vaccinated s.c. with Ag-loaded DCs was similar to that of control mice vaccinated with PBS, and for this reason we chose to vaccinate mice via the i.v. route. The lack of a protective response observed in mice vaccinated with the subcutaneous route is in keeping with the findings of Kuribayashi et al. (52). They found that the Th1/Th2 cytokine profiles induced following subcutaneous administration of Ag-pulsed DCs were different from those induced by DCs administered i.v. The IL-4 levels (protein and RNA) in the spleen cells of mice were higher in mice that received DCs s.c. Similarly, the mRNA levels of IL-4 and IL-5 in the lymph nodes cells was higher in mice that received DCs s.c. IFN-γ protein and mRNA levels were similar following either route of DC administration. Taken together, it appears that s.c. administration of DCs induces a dominant Th2 phenotype. In agreement with Kuribayashi et al. (52), we show that i.v. injection of DCs induces a dominant Th1 phenotype. Furthermore, we show that this phenotype is associated with a reduction in parasite burden.

Despite its appeal, several limitations exist in the use of microbial Ag-pulsed and/or cytokine-transduced autologous DCs in the management of infectious diseases. First, although the techniques for generating large numbers of autologous DCs are in place, the process is laborious and expensive. This limits the ready use of DC-based anti-infective therapy in a developing nation where infections with intracellular pathogens such as Leishmania or M. tuberculosis are common. Second, such an approach may not be feasible in a large-scale setting because the DCs would have to be isolated from individual patients or the naive host. Nevertheless, we have outlined several clear-cut situations where the future use of DC-based anti-infective strategies could be highly beneficial.

In summary, DC-based anti-infective vaccines and therapies comprise a novel yet nascent and embryonic field. Despite potential limitations, our findings provide additional rationale for the future development of DC-based strategies for the prevention and treatment of serious intracellular infections. We also demonstrate that the magnitude and kinetics of the immune response to Ag-pulsed DCs can be enhanced by the paracrine delivery of IL-12 via DCs.

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References


