Efficacy of Antigen 2/Proline-Rich Antigen cDNA-Transfected Dendritic Cells in Immunization of Mice against *Coccidioides posadasii*

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Efficacy of Antigen 2/Proline-Rich Antigen cDNA-Transfected Dendritic Cells in Immunization of Mice against *Coccidioides posadasii*\(^1,2\)

Shanjana Awasthi,\(^3\)* Vibhudutta Awasthi, † D. Mitchell Magee,‡ and Jacqueline J. Coalson*

*Coccidioides posadasii* causes coccidioidomycosis, or Valley fever, in the endemic regions of the Southwestern United States. The susceptibility to *C. posadasii* infection has been attributed to a decreased Th1 cellular response. APCs, especially dendritic cells (DCs), play an important role in the activation of Th1 response. In this study, we investigated the efficacy of a DC-based vaccine against *C. posadasii* in a mouse model of coccidioidomycosis. We intranasally immunized C57BL6 mice with syngeneic, bone marrow-derived DCs (JAWS II cells) transfected with a cDNA encoding the protective *Coccidioides*-Ag2/proline-rich Ag. The immunized mice were lethally challenged with *C. posadasii* through either an i.p. or intranasal route. Upon necropsy after 10 days of infection, fungal burden in lung and spleen of immunized mice was significantly reduced as compared with the control animals. The lung tissue homogenates of immunized animals showed higher levels of IFN-\(\gamma\). Histologically, lung tissues of immunized mice were in better condition than the control mice. To further investigate, we studied the biodistribution and trafficking of injected DCs by nuclear imaging techniques. For this purpose, the transfected DCs were radiolabeled with \(^{111}\)In-oxime. Scintigraphic images showed that most of the label remained in the gastrointestinal tract. A significant amount was also observed in lung, but there were negligible circulating \(^{111}\)In label in blood. The results suggest that the DCs have a potent immunostimulatory activity, and immunization with DCs transfected with Ag2/proline-rich Ag-cDNA induces protective immunity against *C. posadasii* in C57BL6 mice. *The Journal of Immunology*, 2005, 175: 3900–3906.

Due to the virulent nature of *Coccidioides*, endemicity of infection and frequent relapse after chemotherapy, an urgent need for the development of an effective therapy against coccidioidomycosis has been acknowledged (1, 5). It has been established that the protective immunity against *Coccidioides posadasii* is mounted by a Th1 cell response (6, 7). Among different mouse strains, BALB/c and C57BL/6 mice are susceptible to *Coccidioides*, whereas DBA/2 mice are resistant to *Coccidioides* infection (8). The genetic basis of this difference is not clear, but it appears to be associated with depressed Th1 cell reactivity (6, 9). For an efficient protective response, the Ags are required to be carried to the lymph nodes where subsequent activation of naive lymphocytes occurs. Dendritic cells (DCs)\(^5\) are the APCs, which are specialized in capturing the Ags and then migrating to the lymph nodes (10). These unique capabilities of DCs have been recently used to design immunotherapy against cancers and infectious diseases (11–13). The induction of immunity after immunization with DCs depends on the factors such as route of administration, cell-homing, Ag, and phenotype of DCs (10, 14).

A prerequisite to a successful DC-based vaccine is the identification of a potent Ag for in vitro stimulation of DCs. Specific to *Coccidioides*, different Ags have been tested in combination with a variety of adjuvants (15–18). Among these, Ag2/proline-rich Ag (Ag2/PRA) has been identified as having a potent antigenicity (19, 20). In this work, we studied the immunostimulatory potential of syngeneic DCs that had been transfected with Ag2/PRA cDNA. We found that the immunization conferred protection in mice against *Coccidioides* infection. We further imaged trafficking of intranasal \(^{111}\)In-labeled DCs and found that the instilled DCs remained localized in the gastrointestinal tract (g.i.t.) for a prolonged

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\(^2\) Part of this work has been presented by S.A., D.M.M., and R.A. Cox at the Experimental Biology meeting held in Washington, D.C., April 17–21, 2004; by S.A. and V.A. at the Southwest Chapter Society of Nuclear Medicine meeting held in San Antonio, TX, March 14–16, 2005; and by V.A. and S.A. at the Society of Nuclear Medicine meeting held in Toronto, Canada, June 18–22, 2005.

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\(^5\) Abbreviations used in this paper: DC, dendritic cell; PRA, proline-rich Ag; CT, computed tomogram; D-PBS, Dulbecco’s PBS; g.i.t., gastrointestinal tract; SPECT, single photon emission computed tomogram.
period. The accumulation of radioactivity in lung was moderate but sustained.

Materials and Methods

**Mice**

The studies were approved by the Institutional Animal Care and Use Committee and Environmental and Health Safety Committee. C57BL6 mice (6 wk old; The Jackson Laboratory) were housed at the Laboratory Animal Resources in the University of Texas Health Science Center at San Antonio (UTHSCSA). They were provided food and water ad libitum. The mice that were infected with *Coccidioides* were housed in a biosafety level-3 facility. The animals receiving radiolabeled DCs were housed in isolation during the study.

**Transfection of DCs with Ag2/PRA cDNA**

An immortalized dendritic JAWS II cell line derived from bone marrow of C57BL6 mice was obtained from American Type Culture Collection. The Ag2/PRA cDNA was ligated to a pVR1012 vector containing VP22 insert from pVPP22/myc-His (Invitrogen Life Technologies). Vector plasmid pVR1012 containing VP22 insert was used as a control. The cells were transiently transfected with 1.0 μg of DNA per 1 × 10⁶ cells using TransIT-TKO (Mirus) (21). The transfected cells were maintained in complete α-MEM containing 10% FBS, 4 mM L-glutamine, 5 mg/ml recombinant mouse GM-CSF, 100 μg/ml penicillin, 100 U/ml streptomycin, and 50 μg/ml gentamicin. After 24 h, the cells were collected and washed with low-endotoxin Dulbecco’s PBS (D-PBS). Viability of the transfected cells was assessed by trypan blue dye exclusion. Finally, the cells were counted and suspended in D-PBS (33–50 million per milliliter). The expression of Ag2/PRA protein in transfected cells was confirmed by dot-immunoblot assay. Briefly, cell homogenates in a buffer containing protease inhibitors were loaded on a prewetted nitrocellulose membrane and blotted against anti-Ag2/PRA Ab; recombinant Ag2/PRA Ag was run as a control (21).

**Flow cytometric analysis**

The percentage of transfected cells was studied by flow cytometric analysis after staining with fluorochrome-conjugated Abs against various cell surface markers. The cells (1 × 10⁶ per 100 μl of D-PBS with 1% FBS) were incubated with 1 μg of rat anti-mouse CD16/CD32 Ab per 15 min. The cells were then stained with FITC-labeled rat anti-mouse CD4, PE-labeled hamster anti-mouse CD80, PE-labeled hamster anti-mouse CD86, PE-labeled rat anti-mouse CD86, biotin-conjugated rat anti-mouse CD40, or biotin-labeled rat anti-mouse MHC class II Abs (all from BD Pharmingen). After 30 min, the cells were incubated with streptavidin-allophycocyanin conjugate. Appropriate isotype-matched control Abs were used to determine the background level. The fluorescence analysis was performed using a FACSCalibur flow cytometer (BD Immunocytometry Systems). The histogram charts were analyzed using CellQuest version 3.1 software provided with the system.

**C. posadasi**

All experiments with *C. posadasi* were performed in a biosafety level-3 facility. *C. posadasi* strain Silveira was cultured on 1% glucose-0.5% yeast extract agar plates. The arthroconidia were harvested in endotoxin-free 0.15 M saline (Baxter) from the 6- to 8-wk-old mycelial-phase cultures. The arthroconidia suspension was used for infection, and their lungs and spleens were collected. A 10-fold dilution of the tissue homogenates in saline was inoculated on mycobiotic agar plates (Difco) and incubated at 33°C. The number of mycellial colonies were counted and normalized with Gram-weight of tissues.

Lung histology samples were also collected from the animals on the 10th day postinfection. A tissue piece was fixed in 10% formaldehyde. Sections (4 μm) were cut and stained with H&E stain. The histological slides were randomly studied in a blinded fashion and the pathological changes were graded as follows: grade 0—1, no inflammation or patchy areas of inflammation, no organisms; grade 2, small areas of bronchial and peribronchiolar inflammation with few organisms and no necrosis; grade 3, inflammation and necrosis with many organisms in bronchial and peribronchiolar regions.

**Measurement of INF-γ**

A portion of lung tissue was homogenized in a buffer (50 mg of tissue in 1 ml of buffer) containing 1% Igepal CA-630, 0.01% SDS, 1 mg/ml Leu- peptin, 1 mM EDTA, 0.9 mg/ml pepstatin, and 0.2 mM PMSF. The homogenates were filtered through 0.2-μm filters and the INF-γ in this assay was measured by ELISA (BD Pharmingen). Briefly, Immulon 2 plate wells (Dynatech Laboratories) were coated overnight at 4°C with 0.1 μl of purified anti-mouse INF-γ Ab (5 μg/ml; BD Pharmingen) diluted in 0.1 M NaHCO₃, pH 8.2. The coated wells were washed with PBST (0.05% Tween 20 and blocked with PBS containing 3% BSA. The wells were incubated with the homogenate supernatants or standard solutions of recombinant mouse INF-γ (BD Pharmingen). After overnight incubation, biotinylated anti-mouse INF-γ Ab (100 μl, 2.5 μg/ml) was added. The wells were further incubated with streptavidin-peroxidase conjugate, followed by tetramethylbenzidine substrate solution. The reaction was stopped by adding 50 μl of 0.2 N sulfuric acid, and the plates were read at 450 nm. The limit of detection for INF-γ in this assay was 10 pg/ml.

**Radiolabeling and trafficking of transfected DCs**

To elucidate the distribution of intranasally administered DCs, the transfected cells were radiolabeled with a radiochemical [111In]oxime (Amer sham). Briefly, the transfected DCs were gently scraped, twice washed, and suspended in 1 ml of PBS. [111In]-Oxime (1 ml, 1 ml) was added and washed twice with D-PBS. Radiolabeled pellet of cells was resuspended in D-PBS (33–50 million cells per milliliter). A 30-μl aliquot of [111In]-labeled DCs was administered in mice intranasally as described above. The animals were imaged using a small animal XPECT-CT system (Gamma Medica) at 6, 24, and 48 h after instillation. Both single photon emission computed tomograms (SPECT) as well as computed tomograms (CT) were acquired. During imaging, the animals were under isoflurane anesthesia (1% in L/min oxygen). Anatomic locali zation of radioactivity was performed by fusing the SPECT images with the CT images using software provided with the system. For biodis tribution studies, five mice each were sacrificed at 6, 24, 48, and 72 h after administration of radiolabeled DCs. Various organs were harvested, and the associated radioactivity was detected in an automated gamma counter (PerkinElmer). The percentage of radioactivity accumulation in various tissues was calculated on a per Gram tissue basis.

**Statistical analysis**

The results were analyzed by Student’s *t* test or Mann-Whitney *U* test using Prism software (GraphPad). The *t* test was used when the data were normally distributed; the rest were analyzed by Mann-Whitney *U* test.

**Results**

**Characteristics of transfected DCs**

DCs were transfected with Ag2/PRA cDNA with an efficiency of 30–50%, and the transfection was stable for at least 72 h (Fig. 1a). The cells expressed Ag2/PRA protein (Fig. 1b). Approximately 70% of transfected cells were viable at the time of

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immunization. Flow cytometry demonstrated a moderate increase in the expression of MHC class II, CD11c, CD40, CD80, and CD86 on the cell surface of transfected DCs as compared with nontransfected DCs (Fig. 2). The transfected cells were also radiolabeled with $^{111}$In-oxime to visualize in vivo trafficking of DCs. There was no change in the morphology and viability of cells after radiolabeling with $^{111}$In-oxime. Flow cytometry also showed no alteration in the fluorescence intensity values of positive-gated radiolabeled cells as compared with the nonlabeled ones (Fig. 2d).

**Immunization of mice with Ag2/PRA cDNA-transfected DCs**

The immunized mice were infected by live *Coccidioides* arthroconidia through either intranasal or i.p. administration (Table I). The fungal burden in lung and spleen was estimated by culturing the tissue homogenates. Based on the fungal colony counts, the mice immunized with Ag2/PRA cDNA-transfected DCs showed enhanced clearance of fungus as compared with the control mice immunized with vector plasmid-transfected DCs, nontransfected DCs, or Ag2/PRA cDNA alone (Table I; $p < 0.001$, $p < 0.01$, or $p < 0.05$, Mann-Whitney U test). Immunization with a lesser number of Ag2/PRA cDNA-transfected DCs did not induce reduction in fungal load (data not shown). The fungal colony counts were also lower in lung and spleen tissues of the mice immunized with Ag2/PRA cDNA alone. However, the relative fungal burden indicated that the protective effect of Ag2/PRA cDNA was enhanced when DCs were used as an adjuvant (Table I). Interestingly, despite the larger arthroconidia load with the i.p. infection compared with the intranasal infection (2500 vs 30), the reduction in fungal load was more pronounced in lungs of immunized mice challenged i.p. than in lungs of mice infected intranasally.

As an indication of lymphocyte activation, we also estimated IFN-$\gamma$ in the lung homogenate of animals receiving Ag2/PRA cDNA-transfected DCs or vector plasmid DNA-transfected DCs (Fig. 3). The amounts of IFN-$\gamma$ were found to be increased significantly in lung tissue homogenates from mice immunized with Ag2/PRA cDNA-transfected DCs as compared with the homogenates from mice immunized with vector plasmid DNA-transfected DCs ($p < 0.05$, $t$ test). The increase was similar in both i.p. and intranasal infection. The lung histology indicated that the mice immunized with Ag2/PRA cDNA-transfected DCs presented with less tissue damage (grade 0–1 and 2), whereas tissue samples from mice immunized with vector plasmid DNA-transfected DCs showed grade 3 changes of multiple foci of necrosis, chronic inflammation, and many organisms (Figs. 4 and 5). Moreover, the lung tissue damage was significantly less when mice were infected i.p. (all with grade 1 injury) as compared with the intranasal injection (grade 1–3). Thus, the histology results corroborated with the observed reduction in fungal burden in immunized mice.
To investigate the in vivo trafficking of DCs after intranasal immunization, we radiolabeled transfected DCs with $^{111}$In in order to track their movement in the body. The radiolabeling efficiency averaged $\sim 60\%$ and the viability of cells was not affected by the radiolabeling procedure. When the radiolabeled cells were cultured for up to 72 h under standard conditions, there was $<20\%$ loss of label in the media. This showed that the radiolabel was stably associated with the cells. The radiolabeled cells were administered intranasally in mice. The gamma camera images of animals showed pronounced accumulation of cells in the lungs of immunized mice. The accumulation in the lungs persisted for up to 48 h with negligible change in location (Fig. 6). There was a moderate accumulation of radioactivity in the lungs while all the other organs exhibited negligible localization. An overlying CT image was simultaneously acquired in the same animal and was helpful in anatomically localizing the radioactivity in the animals. Similar observations were made in biodistribution experiments when the animals were sacrificed at 6, 24, 48, and 72 h after intranasal administration (Table II). Again, most of the radioactivity was seen associated with the upper g.i.t., i.e., esophagus and stomach. With time, g.i.t.-associated radioactivity rapidly decreased to background level. It appears that the g.i.t. radioactivity gradually cleared in the feces. No attempt was made to separate contents of the g.i.t. Lung, the other organ of major accumulation, showed relatively slower clearance of radiolabeled cells, and even after 72 h, $\sim 23\%$ of initial activity (at 6 h) was present in the lung tissue. Other tissues, including blood, did not show any accumulation of radiolabeled cells at any time point.

**Biodistribution and imaging**

Significant efforts have been made to develop a vaccine against *Coccidioides* during recent times. Various coccidoidal Ags have been identified as potential candidates including a spherule outer wall glycoprotein (22), a *Coccidioides* alkali-soluble, water-soluble extract (23) and $\beta$-1,3-glucan synthetase (24). A killed spherule vaccine was shown to protect the mice and other animals from experimental *Coccidioides* infection, but failed to induce protection in humans in preclinical trials (25). The cell wall glycoprotein Ag2/PRA is the most extensively studied vaccine candidate against coccidioidomycosis. Different groups of investigators have found some success in the resolution of infection after immunization with Ag2/PRA using commercially available adjuvants (19, 20, 26, 27). Reports on efficacy of naked Ag2/PRA cDNA-based vaccine are inconsistent (19, 27, 28). Differences in route of immunization, route of challenge, numbers of arthroconidia, animal model, etc., make it difficult to compare the results from one study to another. It has been advocated that the genetic immunization and autologous DCs have many advantages over immunization with protein Ag and commercial adjuvants, respectively (29).

DCs are professional APCs capable of both priming a Th1-dependent immune reaction and efficiently stimulating memory response. Although present in small numbers, they are particularly localized in skin and mucosal surfaces (30). Thus, it makes sense to populate mucosal surfaces with Ag-pulsed DCs to confer Ag-specific immunity. DCs have been shown to phagocytose and present Ags from several pulmonary pathogens such as *Mycobacterium tuberculosis* (31) and fungal pathogens such as *Aspergillus* (32) and *Candida albicans* (33). Recently, introduction of conidia- or fungal-RNA-transfected DCs was found to induce protective

**Discussion**

<table>
<thead>
<tr>
<th>Immunization</th>
<th>Ag2/PRA cDNA-Transfected DCs</th>
<th>Ag2/PRA cDNA</th>
<th>Vector Plasmid-Transfected DCs</th>
<th>Nontransfected DCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung (i.p.)</td>
<td>2.0 ± 1.4&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>44.0 ± 35.0</td>
<td>45.0 ± 22.0</td>
<td>120.0 ± 92.0</td>
</tr>
<tr>
<td>Spleen (i.p.)</td>
<td>18.0 ± 7.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>34.0 ± 7.5</td>
<td>96.0 ± 34.0</td>
<td>110.0 ± 71.0</td>
</tr>
<tr>
<td>Lung (i.n.)</td>
<td>110.0 ± 24.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>200.0 ± 62.0&lt;sup&gt;f&lt;/sup&gt;</td>
<td>430.0 ± 130.0</td>
<td>490.0 ± 140.0</td>
</tr>
<tr>
<td>Spleen (i.n.)</td>
<td>0.2 ± 0.05&lt;sup&gt;g,h&lt;/sup&gt;</td>
<td>2.3 ± 1.6</td>
<td>1.5 ± 0.8</td>
<td>1.0 ± 0.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> p < 0.05 vs vector plasmid-transfected DCs.
<sup>b</sup> p < 0.01 vs nontransfected DCs.
<sup>c</sup> p < 0.001 vs Ag2/PRA cDNA alone.
<sup>d</sup> p < 0.001 vs vector plasmid-transfected DCs.
<sup>e</sup> p < 0.05 vs nontransfected DCs.
<sup>f</sup> p < 0.05 vs Ag2/PRA cDNA alone.
<sup>g</sup> p < 0.05 vs vector-plasmid-transfected DCs.
<sup>h</sup> p < 0.005 vs Ag2/PRA cDNA alone.

**Table I.** Fungal burden (10<sup>4</sup> cfu/g tissue, mean ± SEM) in immunized mice after intranasal (i.n.) and i.p. infection with *C. posadasii* arthroconidia.
response in vivo against C. albicans, Aspergillus fumigatus, and Cryptococcus neoformans (34–36). It has also been shown that peripheral blood-derived DCs, pulsed with a soluble extract of Coccidioides immitis spherules, can prime lymphocytes in vitro (37). Another ensuing study demonstrated that autologous, C. immitis Ag-pulsed DCs could reverse the in vitro anergic T cells response against C. immitis in patients with disseminated coccidioidomycosis (38). In Coccidioides infection, activated Th1 response is responsible for protective acquired immunity, and DCs may play an important role in connecting the innate immune response to the adaptive Th1 immunity (37, 38). These studies suggest that DC-based immune therapy could be useful in the treatment of disseminated coccidioidomycosis.

Although viral transfection reagents are the mainstay in eukaryotic cell transfection, it has its own immunological drawbacks, and attempts are being made to replace them with novel nonviral reagents that can produce high transfection efficiency (39). We recently showed that TransIT-TKO reagent can be successfully used to transfect DCs with 30–50% transfection efficiency (21). Using the same technique, we transfected bone marrow-derived DCs and intranasally immunized mice to induce C. posadasii-specific immune response. Because lung is the first organ that encounters inhaled arthroconidia, our primary objective was to monitor lung pathology and microbial burden. We hypothesized that enhancing the C. posadasii-specific response in the lung may be beneficial against subsequent Coccidioides infection. Our results show that the immunization with Ag2/PRA cDNA-transfected DCs induces protective immunity and reduces fungal burden in C. posadasii-susceptible C57BL6 mice against a lethal challenge with C. posadasii. It appears that intranasal immunization confers immunity against infection from both pulmonary as well as i.p. route. A significant immunity was also observed when naked Ag2/PRA cDNA was administered, suggesting an important role of the components of mucosal barriers in immunological response against pulmonary infection. However, the protective effect is significantly enhanced when the immunization is performed with DCs as an adjuvant.

Quantitatively, compared with control mice, fungal burden in the lung tissue was reduced by a factor of 100 in immunized mice that were challenged i.p.; in the intranasal infection of immunized mice.

Table II. Distribution of $^{111}$In label in mice following intranasal instillation of $^{111}$In-labeled DCs

<table>
<thead>
<tr>
<th>Organ</th>
<th>Percent Accumulation per Gram of Tissue (Mean ± SEM)</th>
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<tr>
<td></td>
<td>6 h</td>
</tr>
<tr>
<td>Blood</td>
<td>0.21 ± 0.04</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>Trachea</td>
<td>0.39 ± 0.33</td>
</tr>
<tr>
<td>Lung</td>
<td>11.36 ± 3.44</td>
</tr>
<tr>
<td>Liver</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>Esophagus and stomach</td>
<td>55.11 ± 5.96</td>
</tr>
<tr>
<td>Intestine</td>
<td>9.92 ± 1.79</td>
</tr>
</tbody>
</table>

*p < 0.05 vs 6 h.
mice, the fungal reduction only occurred by a factor of 5 (Table I). The immunized mice also demonstrated better histological grading when they were i.p. infected as compared with the intranasal infection (Figs. 4 and 5). Perhaps a majority of nasally administered DCs travel to the gut, bypassing the trachea. To investigate this possibility, we radiolabeled transfected DCs with a gamma-emitting radionuclide \(^{111}\)In using an oxime complex. Apparently, radiolabeling does not alter the phenotypic properties of transfected DCs (Fig. 2). When intranasally administered, \(>65\%\) label was found to accumulate in the g.i.t comprising of esophagus, stomach, and intestine, while pulmonary accumulation was relatively moderate (11.4%). The radioactivity disappeared from the g.i.t. within 48 h of administration, but \(\geq 23\%\) of pulmonary radioactivity remained in the lung even after 72 h. The observation that the radioactivity was not seen in the systemic circulation, spleen, and other organs suggests that the intranasal DCs are capable of locally performing their function.

Migration of endogenous DCs is dependent on their interaction with the endothelial adhesion molecules and the chemokines produced at the local microenvironment (30). Once in the tissue, the DCs process the microbial Ags and attain maturity that is characterized by a down-regulation of chemokine receptors and an up-regulation of CCR7. The latter confers lymph node homing capability to the mature DCs (40). The current understanding of lymphatic homing of DCs is based on skin as a model. The mechanisms by which pulmonary or gut pathogen are monitored by the DCs are largely unknown (30). Although it has not been possible to demonstrate the direct involvement of pulmonary or gut lymphatics, the fact that the immunized mice showed protection against both i.p. and intranasal infection supports this conjecture. The scintigraphic images provide important visual impressions of the linear migration of cells in temporal fashion.

One drawback of this study is the use of bone marrow-derived DCs rather than the pulmonary DCs. Isolation of pulmonary DCs is difficult because they constitute \(<1\%\) of total cells (41). Bone marrow is a rich source of DCs and has been used successfully for vaccine development and immunotherapy (11). In a clinical situation, bone marrow-derived DCs would be easier to obtain than the lung DCs. Therefore, we used well-characterized, immortalized C57BL6 mouse bone marrow-derived JAWS II cells. Another limitation of this study is the lack of any direct information on in vivo interaction of the DCs with naive lymphocytes leading up to the activation of the latter. Monitoring IFN-\(\gamma\) in lung tissue partially mitigates this limitation. IFN-\(\gamma\) is a major IFN produced by mitogenically or antigenically stimulated lymphocytes and considered as a direct indicator of lymphocyte activation (Fig. 3). Our findings that the immunization with Ag2/PRA cDNA-transfected DCs induces the protective immune response and reduces the fungal burden after lethal arthrocondia challenge, provides support for a role of DCs in the development of vaccine against C. posadasii.

Acknowledgments

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Disclosures

The authors have no financial conflict of interest.

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