Molecular Basis for the Potency of IL-10-Deficient Dendritic Cells as a Highly Efficient APC System for Activating Th1 Response

Qing He, Terri T. Moore, Francis O. Eko, Deborah Lyn, Godwin A. Ananaba, Amy Martin, Shailesh Singh, Lillard James, Jonathan Stiles, Carolyn M. Black and Joseph U. Igietseme


http://www.jimmunol.org/content/174/8/4860

**References**

This article cites 64 articles, 31 of which you can access for free at: http://www.jimmunol.org/content/174/8/4860.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Molecular Basis for the Potency of IL-10-Deficient Dendritic Cells as a Highly Efficient APC System for Activating Th1 Response

Qing He,*† Terri T. Moore,* Francis O. Eko,* Deborah Lyn,* Godwin A. Ananaba,‡ Amy Martin,† Shailesh Singh,* Lillard James,* Jonathan Stiles,* Carolyn M. Black,† and Joseph U. Igietseme2*†

Identification and targeting of novel immunobiological factors that regulate the induction of Th1 cells are crucial for designing effective vaccines against certain intracellular pathogens, including Chlamydia. IL-10-deficient dendritic cells (DC) are potent APCs and effective cellular vaccines that activate a high frequency of specific Th1 cells. To elucidate the molecular basis for the potency of the IL-10-deficient APC system, we tested the hypothesis that Chlamydia Ag-primed IL-10 knockout (IL-10KO) DC are quantitatively and qualitatively distinct in their metabolic characteristics relating to T cell activation. Using a combination of RT-PCR, two-dimensional gel electrophoresis, and MALDI-TOF-based proteomics analyses, the transcriptional and translational activities of Chlamydia-pulsed DC from wild-type and IL-10KO mice were assessed. IL-10 deficiency caused early maturation and activation of pulsed DC (i.e., high CD11c, CD40, CD80, CD83, CD86, IL-1, IL-12, and the T cell-attracting chemokine CCL27/CTACK) and consequently an enhanced ability to process and present Ags for a rapid and robust T cell activation. Supporting comparative proteomics revealed further that IL-10 deficient DC possess specific immunobiological properties, e.g., the T cell-attracting chemokine CCL27/CTACK, calcium-dependent protein kinase, and the IL-1/IL-12 inducer, NKR-P1A (CD161), which differentiated them immunologically from wild-type DC that express molecules relating to anti-inflammatory, differentiative, and metabolic processes, e.g., the anti-IL-12 molecule peroxisome proliferator-activated receptor-α and thymidine kinase. Collectively, these results provide a molecular basis for the high Th1-activating capacity of IL-10KO APC and may provide unique immunomodulation targets when designing vaccines against pathogens controlled by T cell immunity. The Journal of Immunology, 2005, 174: 4860–4869.

A
n efficacious vaccine is the best approach to protect the greatest number of people from the mucosal and systemic infections caused by several intracellular microbial pathogens that include Chlamydia. For several intracellular microbial pathogens, protective immunity in humans as well as animals is governed by the elicitation and maintenance of a high level of T cell-mediated immune response (1–7). The notable effectors of T cell-mediated immunity (CMI) are CTLs, which are mostly CD8 T cells; Th1 cells, commonly CD4 T cells; inflam-

*multiple affiliations

1 This work was supported by Department of Health and Human Services Grants AI41231, GM08247, GM08248, and RR03034 from the National Institutes of Health with 18 U.S.C. Section 1734 solely to indicate this fact.
2 Address correspondence and reprint requests to Dr. Joseph U. Igietseme, National Center for Infectious Diseases/Scientific Resources Program/Centers for Disease Control and Prevention, Atlanta, GA 30333. E-mail address: jigietseme@cdc.gov
3 Abbreviations used in this paper: CMI, T cell-mediated immunity; IL-10KO, IL-10 knockout; CTACK, cutaneous T cell-attracting chemokine; 2-DE, two-dimensional gel electrophoresis; DC, dendritic cell; EB, elementary body; WT, wild type; IFU, inclusion-forming unit; RT, reverse transcription; IEF, isoelectric focusing; pl, isoelectric point; MSP, macrophage-stimulating protein; RON, tyrosine kinase/recepteur d’origine nantais; PD-1, programmed cell death-1 ligand.

Copyright © 2005 by The American Association of Immunologists, Inc.

The Journal of Immunology
now better appreciated that the cytokine environment at the initiation of T cell response and the magnitude of the concurrent humoral immune response exert significant regulatory influence on the level and effectiveness of T cell immunity against intracellular microbial pathogens (8). Among the cytokines required, IL-12 derived from the APCs (18) is critical for Th1 response. TLRs, a family of microbial pattern recognition receptors expressed on APCs, play a major role in sensing the presence of microbial pathogens. They consequently stimulate the maturational activation of APCs to acquire enhanced Ag processing and presentation capacity that includes IL-12 production (19–22). IL-1 and IL-18 are also potent inducers of Th1 response and are components of the cytokine environment that favor Th1 activation. IL-18 knockout mice are deficient in both NK cell function and Th1 development, and IL-18 is vital for antifungal immunity (7, 23). It is therefore conceivable that specific vaccine delivery systems can incorporate the concurrent administration of certain cytokines and vaccine to produce an ambient cytokine environment for optimal induction of protective Th1 response against microbial pathogens. Furthermore, the biological suppression of certain cytokines may promote Th1 induction and thereby enhance protective immunity against certain microbial pathogens. IL-10, an anti-Th1 cytokine (24) produced during genital chlamydial infection of experimental animals and humans (25, 26), is potentially an important local factor that would control Th1 response against Chlamydia. Thus, genetic differences in mouse strain susceptibility to Chlamydia trachomatis or Coccidioides immitis was mapped to high IL-10 producers being more susceptible to infections (25, 27). Also, increased IL-10 levels in the endovascular secretion of women with chlamydial infection could predispose to enhanced HIV-1 transmission (26), possibly due to inadequate local T cell response. These observations would suggest that the temporary or inadequate immunity commonly induced against Chlamydia is at least partially due to the negative immunoregulation of IL-10, which could occur via IL-10-regulated production of a relatively low frequency of Chlamydia-specific Th1 cells. In such a case, genetic or biological suppression of IL-10 activity or expression during immune elicitation against Chlamydia should lead to the induction of high levels of specific Th1 response that could mediate long term antichlamydial immunity. Indeed, microbiological analysis of the role of IL-10 in resistance against Chlamydia using genetically engineered IL-10KO mice revealed that the animals were more resistant to genital and respiratory infection than wild types, which correlated with the high Th1 response (25, 28, 29). Also, the cellular and molecular basis for the high Th1 response in IL-10KO mice was shown to be due to the proclivity of IL-10KO APC for activating Th1 cells regardless of whether the T cells express IL-10, suggesting that the effect of IL-10 is exerted on the APC (28). In addition, Chlamydia-pulsed IL-10KO dendritic cells (DC) were efficient cellular vaccines, inducing high frequencies of systemic and mucosal Th1 response in recipients that acquired sterilizing, long term protective Th1 response against Chlamydia (28). These findings are corroborated by other reports that IL-10 affects DC maturation and APC function (30–35) and promotes tumor growth by suppressing T cell response (36, 37). In this study, we used transcriptional and proteomics techniques to analyze the immunobiological features of Chlamydia-exposed IL-10-deficient DC to determine the molecular and biological basis of the high Th cell activating capacity of this efficient APC system. The results from these studies could lead to the identification of ligand-sensitive pathways that can be rationally manipulated during vaccine delivery to obtain an optimal protective immunity against intracellular microbial pathogens.

### Materials and Methods

**Chlamydia stocks and Ags**

Stocks of C. trachomatis serovar D and Chlamydia muridarum (the agent of mouse pneumonitis) used for infections were prepared by propagating elementary bodies (EBs) in McCoy or HeLa cells, according to standard procedures. Chlamydial stock titers were expressed as inclusion-forming units (IFU) per milliliter. Chlamydial Ags were prepared by growing the agent in HeLa cells and purifying EBs over Renografin gradients, followed by inactivation under UV light for 3 h.

**Knockout and wild-type (WT) mice**

Female IL-10−/−, IFN-γ−/−, and the respective control IL-10+/+ and IFN-γ+/+ mice, on a C57BL/6 background and 5–8 wk old, were obtained from The Jackson Laboratory. All animals were fed with food and water ad libitum and maintained in laminar flow racks under pathogen-free conditions of 12 h light and 12 h darkness.

**DC isolation and culture**

DCs were isolated from the bone marrows of normal and IL-10KO mice by the standard method and differentiated by in vitro culture with IL-4 and GM-CSF, as previously described (28). The cells were characterized as loosely adherent mononuclear cells and determined by FACS to express high levels of MHC class II, CD54 (ICAM-1), and CD11c. After 5 days in culture, DCs were washed and pulsed with UV-inactivated Chlamydia EBs.

**Adaptive immunotherapeutic vaccination with Chlamydia-pulsed DCs**

DCs isolated from IL-10−/− and IL-10+/+ were pulsed with UV-inactivated C. trachomatis serovar D EBs for 12 h and adoptively transferred into 4-wk-old homogenic, female IFN-γ−/− and the control IFN-γ+/+ mice (2.5 × 105 cells/mouse), by i.v. infusion into the retro-orbital sinus in 0.2 ml of PBS. Treated mice were maintained in a laminar flow hood, fed, and maintained under a 12-h light:12-h dark cycle. After 1 week, the mice were infected intravaginally with 104 IFU/mouse of live C. trachomatis serovar D. The status of the infection was monitored by periodic cervicovaginal swabbing of individual animal and isolation of chlamydial in tissue culture (38). Experiments were repeated twice to give 10–12 mice/group.

**Measurement of frequency of Chlamydia-specific Th1 cells (Th1 frequency) after vaccination with Chlamydia-pulsed DC**

A modified procedure of the limiting dilution technique (39) was used to assess Th1 frequency in infected mice. Briefly, T cells were isolated from the genital tracts of infected mice at the indicated times, as previously described (40). Limiting dilution cultures were established by seeding T cells in a serial doubling dilution into 96-well round-bottom tissue culture plates at 12–24 wells/dilution. Typically, four and eight dilutions were established for each T cell responder in the range of 1 × 103 through 8 × 105 cells/well. The T cells were stimulated with APCs from WT mice (2–105 cells/well) and chlamydial Ag (10 μg/ml). Background cultures contained 24 wells with APCs and Ag. After 5 days of incubation, the supernatants were assayed for IFN-γ by a sensitive ELISA (40). The mean and SD of all 24 replicates of background cultures were calculated. Three times the value of the SD was added to the mean value, and the sum was the baseline for positive experimental wells. After determination of the number of positive and negative wells per dilution of each T cell preparation, the data were analyzed by a limiting dilution computer program (LIDIA) (39, 41), which provided both the Th1 frequency and the conformity of the input data with a single-hit Poisson model. Genital tract T cells from naïve IL-10−/− mice have Th1 frequency of 15 (range, 9–21).

**Measurement of the rate of T cell activation by DC from IL-10−/− and IL-10+/+ mice**

The efficiency of Ag presentation by DCs from IL-10KO mice and WT mice was compared by assessing the rate of Th1 activation by γ-irradiated DC from IL-10KO and WT mice. Spleen cells from chlamydial-infected IL-10KO and WT mice were enriched for T cells by the nylon wool adhesion method (42, 43). Purified splenic cells contained at least 97% CD3+ cells, as determined by FACS. To assess the Ag-presenting function of γ-irradiated DCs from either IL-10KO or control mice, 1 × 104 or 1 × 105 cells were cocultured with 2 × 105 nylon wool-purified T cells in the presence or absence of chlamydial Ag (i.e., UV-inactivated mouse pneumonitis EBs at 10 μg/ml) in 96-well tissue culture plates for 24, 48, 72, 96,
or 120 h. At the end of each incubation period, the supernatants were collected and assayed for IL-2 and/or IFN-γ content by a quantitative ELISA (Cytoscreen Immunoassay Kit; BioSource) according to the supplier’s instructions. The concentration of the cytokine in each sample was obtained by extrapolation from a standard calibration curve generated simultaneously. Data were calculated as the mean values (±SD) of triplicate cultures for each experiment. The results were derived from at least three independent experiments.

Semi-quantitative RT-PCR analysis of mRNAs from Chlamydia-pulsed bone marrow DCs

To measure changes in gene expression of specific transcripts in Chlamydia-exposed IL-10KO and WT DC by semi-quantitative RT-PCR, cultured DCs were pulsed with Chlamydia (multiplicity of infection, 10) at different time intervals representing early (8 h) and late (72 h) exposure. For total RNA isolation using Trizol reagent (Life Technologies), cultured DC were lysed and subsequently homogenized in Trizol (1 ml per 3.5-cm diameter of culture dish). RNA was precipitated, pelletted, and resuspended in diethyl pyrocarbonate-treated, nuclease-free water and stored at 80°C. For reverse transcription (RT), 4 μl of random hexamer (50 μM) were added to each reaction tube containing 5 μg of total RNA in 14.5 μl of sterile water, incubated for 5 min at 70°C, and then quickly chilled on ice. RNA- sin (0.5 μl), 5X RT buffer (10 μl), dNTPs (20 μl), and Moloney murine leukemia virus RT (1 μl) were added, and the reaction tubes were incubated at 37°C for 1 h. The reaction was terminated by heating at 95°C for 10 min followed by quick chill on ice. Sterile water (50 μl) was added to each reaction tube. Subsequently, the cDNAs were amplified using gene-specific primers for IL-1β, IL-10, IL-12 (p40), ICAM-1, CD11c, CD14, CD4, and β2-MG. The parameters used in the search were as follows: peptide mass, isoelectric point, tolerance 50 ppm, 1 missed cleavage, carboxymethylated cysteine, and N-terminal hydroxylation.

Results

Protective Th1 cells have obligatory requirement for IFN-γ to clear chlamydial infection

Initial studies extended previous findings that IL-10-deficient APC have a proclivity for activating a rapid and protective anti-chlamydial Th1 response. We tested the hypothesis that protective Chlamydia-specific Th1 cells induced by IL-10KO DC cleared chlamydial infection in vivo via IFN-γ-induced antimicrobial processes. Chlamydia-pulsed IL-10KO DC were adoptively transferred into female IFN-γ receptor knockout (IFN-γR−/−) and the control IFN-γR−/− mice. The mice were infected intravaginally with the live chlamydial agent after 1 wk of adoptive transfer, and the course of the infection was monitored by isolation of chlamydiae from cervicovaginal swabs. Fig. 1 shows that on day 3 post-challenge, only 1 of 12 control IFN-γR−/− mice that received Chlamydia-pulsed IL-10KO DC revealed evidence of a suspicious low grade (2.0 ± 2 IFU/ml) infection, which was resolved by day 6 (Fig. 1, D). Thus, 11 of 12 IFN-γR−/− mice that received Chlamydia-pulsed IL-10KO DC were immune to challenge infection, and all these mice cleared their infections within 1 week. The specificity of the protective immunity achieved is evident in the results, showing that the course of the genital infection in IFN-γR−/− mice that received non-Chlamydia-pulsed IL-10KO DC
the response of unvaccinated WT mice when the latter resolved the infection by 4 – 6 wk (specific T cell frequency of 286 ± 24 Th1 cells/10^6 genital tract cells). Overall, there was ~15-fold difference in the frequency of Th1 cells induced by the pulsed IL-10KO DC compared with the natural host response during the course of the infection. When the IFN-γR ^+/+ mice that received Chlamydia-pulsed IL-10KO DC (vaccinated group) and the recipients of DC only (control group) were rechallenged after 99 days of the primary infection, the former enjoyed a sterilizing immunity against Chlamydia, whereas 100% of the latter were successfully infected during the first 10 days after challenge (0 of 12 mice infected in the vaccinated group and 10 of 10 mice infected in the control group). Evaluation of the frequency of Chlamydia-specific Th1 cells in the genital mucosa of these reinfected mice revealed that the IFN-γR ^+/+ mice that previously received Chlamydia-pulsed IL-10KO DC had 1256 ± 85 Th1 cell/10^6 genital tract cells, whereas the control group had 16-fold lower levels (76 ± 8). The results corroborate previous findings that the susceptibility to chlamydial reinfection is due to the time-dependent waning of local T cell effectors after the resolution of the initial infection (43). Also, long-term immunity is due to the retention of a high frequency of immune effectors in the mucosal site of infection. In addition, these data support the need for an efficacious vaccine regimen that induces robust immune effectors beyond the level provided by the natural host response to an infection to maintain adequate and effective residual local immune effectors to control chlamydial infection and reinfections. Because the level of Th1 response measured did not appear to cause any discernible overt pathological consequences for vaccinated mice, the results would suggest that the level of T cell response attained was safe for the animals. However, the optimal Th1 response that would be safe for vaccinated host would have to be established in preclinical evaluation of any vaccine regimen against Chlamydia.

**IL-10 deficiency enhances the rate of Th1 activation in vitro**

The general hypothesis derived from the foregoing studies is that IL-10 deficiency leads to a rapid activation of an elevated level of Th1 response but the cellular targets of IL-10 effects must be clarified. Also, previous studies indicated that the endogenous IL-10 deficiency at the level of the APC, not T cells, is crucial for the elevated Th1 activation (28); however, it is unclear whether IL-10 deficiency at the level of the T cells has a role at any stage during the initiation and progression of T cell activation. In the current study, the rate of Th1 development was assessed in the presence or absence of IL-10 at the level of the APC or T cells during the course of T cell activation in vitro. The data shown in Fig. 3 reveal that the differential rate of T cell activation by IL-10KO and WT DC manifests within 24 h of stimulation when there is ~19-fold enhancement in the absence of IL-10. By 48, 72, 96, and 120 h of culture, there were ~12-, 8-, 6-, and 4-fold increases in T cell activation by IL-10KO DC over WT DC, respectively. These results indicated that IL-10KO APC activate a high frequency of T cells very rapidly and reaffirm previous findings that the endogenous IL-10 produced by the APC is a crucial regulatory factor in the rate and magnitude of T cell activation; however, the IL-10 contributed by T cells do not play a discernible role in the regulation of T cell activation by Ag and APC. Thus, further analysis of IL-10 deficient DC could furnish a model APC system for defining the molecular and biochemical determinants for inducing an enhanced T cell response, and the modulation of these factors can lead to more effective vaccines against agents controlled by T cell immunity.
cells were isolated from *Chlamydia* specific Th1 cells by WT and IL-10KO DC. Nylon wool-purified splenic T cells were infected with *Chlamydia* and measured the early transcriptional activities of genes encoding molecules that are known to be involved in APC-T cell interaction during Ag presentation and T cell activation. The second strategy (i.e., Ag-induced proteomics) involves exposing WT and IL-10KO DC to *Chlamydia* Ags and measuring the early transcriptional activities of genes encoding molecules that are known to be involved in APC-T cell interaction during Ag presentation and T cell activation. The second strategy could reveal novel molecular entities that may play a role in the enhanced Th1 activation capacity of IL-10-deficient APCs. We have previously shown that the enhanced APC function of IL-10-deficient DC is evident within 24 h of exposure of T cells to Ag-pulsed DC (Fig. 3 and Ref. 28). Besides, the kinetics of activation of T cells in vitro with Ag-pulsed WT DC indicated that a pulse time of 8 h produced the optimal immunostimulatory DC (45). Thus, the time periods selected for transcriptional and translational analyses reflected these established reports.

When WT and IL-10KO DC were exposed to *Chlamydia* Ags and analyzed by RT-PCR for the expression of specific immunoregulatory molecules, the results revealed that there was a more rapid induction of high levels of CD11c, IL-1β, IL-12, CD40, and CD80 by IL-10KO DCs when compared with WT DCs (Fig. 4, A–E) except CD86 and ICAM-1 (Fig. 4, F and G). Immunoassay analysis of secreted or cell-associated gene products confirmed the differential expression of these immunostimulatory molecules by IL-10KO and WT DCs (data not shown). Immunoassay evaluation also confirmed that IL-10KO DC did not secret significant IL-10 after exposure to *Chlamydia* although the WT DC secreted elevated levels of the cytokine (Fig. 4H). CD11c is a component of the leukocyte integrin α<sub>β</sub> (CD11c/CD18 or a surface receptor CR4) on mature DC that function in cell adhesion, particle uptake, and phagocytic responses (46–48). There was at least a 9-fold increase in the levels of CD11c mRNA expression (8 h) in *Chlamydia*-pulsed IL-10KO DCs as compared with WT DCs. Thus, the rapid and early expression of the marker of DC maturation and activation (CD11c) would suggest that DCs are more rapidly activated as functional APCs by Ags in the absence of IL-10. The results indicated that IL-10 deficiency caused an earlier maturation of APC such as DC, leading to an enhanced capacity to express crucial costimulatory molecules such as IL-1β CD40, and IL-12 that are important for the differentiative activation of T cells along the Th1 pathway.

Proteomic analysis of the differential translational activities in Ag-pulsed IL-10KO and WT DC involved the used of 2-DE and MALDI-TOF techniques. The study objective was to confirm the differential expression of the costimulatory molecules detected by transcriptional analysis described previously (Fig. 4) and to explore the possibility of identifying novel molecular targets for modulating T cell response during vaccine delivery. The time of DC exposure to Ag for proteomic analysis was limited to the first 8 h because this time period was previously shown to sufficiently prime DC for efficient Ag presentation and T cell activation (45). When IL-10KO and WT DC were pulsed with *Chlamydia* EBs for 2 or 8 h, then analyzed by 2-DE, Fig. 5 shows a representative protein profile map revealing that a number of proteins were differentially expressed by IL-10KO DC compared with WT DC.

Table I provides the biological profiles of the more visibly identified and MALDI-TOF-characterized spots in Fig. 5. At least six notable categories of differentially expressed proteins were identified: 1) proteins that were expressed rapidly early (2 h) in IL-10KO DC but delayed (8 h) in WT DC (i.e., B7.1; the T cell-attracting chemokine CCL27/CTACK; macrophage-stimulating protein (MSP) receptor tyrosine kinase/recepteur d’origine natif (RON) involved in leukocyte spreading, migration, and particle uptake; prolly endopeptidase; and a certain selenoprotein); 2) proteins that were expressed rapidly early in WT DC but delayed in IL-10KO DC (G protein γ2, CAMP-dependent protein kinase regulatory subunit RI-α, and calgranulin B S100A9); 3) proteins that were expressed only (early or delayed) in IL-10KO DC but not in WT DC (i.e., α cardiac actin; sodium channel; calcium/calcmodulin-dependent protein kinase that increase CD83 and costimulation; C-kinesin, and NKR-P1 the engagement of which enhances DC function for IL-12 production); 4) proteins that were expressed exclusively (early or late) in WT DCs but not in IL-10KO DCs (i.e., anti-inflammatory programmed cell death-1 ligand (PD-1) and CCR4-NOT complex; vimentin related to macrophage function; MLCK involved in cellular interaction; ribonucleoside-diphosphate reductase prominin-1 T3 isoform; purine nucleoside phosphorylase; caveolin-1; intersectin 6 associated with cell division and marker of immature DC; keratinocyte lipid-binding protein that suppresses inflammation via binding to peroxisome proliferator-activated receptor (PPAR-α); carcoenmyobronic Ag-7; β<sub>2</sub>-adrenergic receptor (which is antiactiavatory); calcium-activated chloride channel-2 associated with cell secretion and division; anti-inflammatory sterol 1; and the transcription factor Sox-10 involved in cell differentiation, glucose-6-phosphate deaminase, an unknown protein (mKIAA1759, molecular mass, 100+ kDa/pcf 5.6; lymphocyte Ag 6 complex involved in differentiation; Elac 1 protein; thymidine kinase; and extracellular matrix protein 1 precursor); 5) proteins that were expressed early at higher (i.e., STAT1, latent transforming growth factor β, Bcl-2, and the GTP-binding protein rho) or lower (the anti-IL-12 molecule PPAR-α levels in IL-10KO DC than WT DC; and 6) proteins that were expressed at equal levels in IL-10KO and WT DC (i.e., Janus kinase (STAT3); and IFNR-class II cytokine receptor, an unknown mKIAA1775 protein; the tripartite motif protein, cal-modulin; CLIP-associating protein-1). A better understanding of the functional aspects of the differentially expressed proteins, through analysis of genetically engineered specific gene knockout systems and the use of immunological and biochemical blockers, may furnish targets for molecular basis for the enhanced Th1 activation by IL-10-deficient APC

Two strategies were adopted to analyze the molecular basis for the potency of IL-10-deficient DC as a highly efficient Th1-activating APC system. The first strategy (i.e., Ag-induced gene expression analysis) involves exposing WT and IL-10KO DC to *Chlamydia* Ags and measuring the early transcriptional activities of genes encoding molecules that are known to be involved in APC-T cell interaction during Ag presentation and T cell activation. The second strategy (i.e., Ag-induced proteomics) involves exposing WT and IL-10KO DC to *Chlamydia* Ags, the separation and analysis of the proteome by 2-DE, and the identification of differentially expressed molecules by MALDI-TOF. The second strategy could reveal novel molecular entities that may play a role in the enhanced Th1 activation capacity of IL-10-deficient APCs. We have previously shown that the enhanced APC function of IL-10-deficient DC is evident within 24 h of exposure of T cells to Ag-pulsed DC (Fig. 3 and Ref. 28). Besides, the kinetics of activation of T cells in vitro with Ag-pulsed WT DC indicated that a pulse time of 8 h produced the optimal immunostimulatory DC (45). Thus, the time periods selected for transcriptional and translational analyses reflected these established reports.
modulating Ag presentation for an enhanced T cell response against microbial pathogens and tumors that require rapid and elevated T cell response for clearance and establishment of long term immunity.

Discussion
A better understanding of the cellular and molecular immunological factors that govern the induction of a robust T cell response against microbes is a prerequisite for designing efficacious vaccines to protect the population against certain intracellular microbial pathogens that include Chlamydia, Listeria, and Mycobacterium. For several of these pathogens, results from analysis of experimental animal models of the diseases and clinical observation from infected patients have established that: 1) a relatively elevated T cell response is crucial for clearance of infection and maintenance of protective immunity against
reinfections; and 2) the immunobiological basis of the partial and transient protective immunity observed after the clearance of an infection or the administration of several experimental vaccines appears to be due to the induction of an inadequate or a low frequency of CMI effectors at the mucosal sites of infection.

In the case of the animal models used for study of the immunobiology of *C. trachomatis* genital infection and vaccine evaluation, immunization regimens that include the use of ex vivo Ag-pulsed DC to deliver and present *Chlamydia* Ags in vivo have produced some of the most promising experimental protection studies to date (28, 49). *Chlamydia*-pulsed DC appear to possess the necessary antigenic, costimulatory, and immunomodulatory machinery for inducing high levels of Th1 response and the accessory IgA and IgG effectors required for boosting protective immunity against *Chlamydia* (28, 49). Although the use of wild-type DC produced a partial but significant protection against genital chlamydial infection, the application of IL-10KO DC achieved a sterilizing, long term protective immunity that correlated with the capacity to induce a high frequency of specific Th1 cells and elevated titers of the CMI-associated IgG2a and IgA Abs (3, 28). The present study has confirmed the proposed correlation between rapid resolution of genital chlamydial infection, acquisition of protective immunity, and a rapid induction of a high frequency of genital mucosal Th1 response by *Chlamydia*-pulsed IL-10KO DC. In addition, IFN-γ produced by the activated Th1 cells was crucial for chlamydial clearance in this model system.

### Table I. Biological profiles of the more visibly identified and MALD-TOF-characterized spots in Fig 5

<table>
<thead>
<tr>
<th>Spot</th>
<th>Molecular Mass (kDa)</th>
<th>pI</th>
<th>Name of candidate proteins</th>
<th>IL-10&lt;sup&gt;+/−&lt;/sup&gt; 2 h</th>
<th>IL-10&lt;sup&gt;+/−&lt;/sup&gt; 8 h</th>
<th>IL-10&lt;sup&gt;−/−&lt;/sup&gt; 2 h</th>
<th>IL-10&lt;sup&gt;−/−&lt;/sup&gt; 8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>150–250</td>
<td>5.3</td>
<td>MSP receptor (RON): macrophage spreading, migration, and phagocytosis</td>
<td>0</td>
<td>422</td>
<td>3205</td>
<td>2981</td>
</tr>
<tr>
<td>2</td>
<td>32.1–39.7</td>
<td>5.4</td>
<td>Programmed cell death 1 ligand 1(PD-1): a new member of the CD28/CTLA-4 family and is undetectable on activated macrophages or DCs</td>
<td>2943</td>
<td>3988</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>7.1–17.5</td>
<td>5.3</td>
<td>1.NKR-P1: increases intracellular calcium and IL-1 β and IL-12 production by DCs. Thus help to present Ags to T cell</td>
<td>0</td>
<td>0</td>
<td>1279</td>
<td>2551</td>
</tr>
<tr>
<td>4</td>
<td>32.1–39.7</td>
<td>4.7</td>
<td>CCR4-NOT complex</td>
<td>2890</td>
<td>4095</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>&lt;7.1</td>
<td>9.7</td>
<td>B7.1: Costimulatory molecule of mature DCs</td>
<td>0</td>
<td>2273</td>
<td>4095</td>
<td>4095</td>
</tr>
</tbody>
</table>

**FIGURE 5.** Proteomic analysis of the Ag-induced gene expression in WT and IL-10KO DC using 2-DE and MALDI-TOF. WT and IL-10KO DC were pulsed with UV-inactivated chlamydial EBs for 2 (A and B) or 8 (C and D) h, respectively. The proteins from *Chlamydia*-pulsed DC were separated by 2-DE over the pH range of 3.0–10. Specific proteins corresponding to selected spots on the gels were identified by MALDI-TOF, as described in Materials and Methods. The numbers refer to the proteins that have been identified and presented in Table I.
basis of the antimicrobial action of IFN-γ has been recently reviewed (see recent review in Ref. 8).

It has been suggested that the phenomenal efficacy of the DC-based cellular vaccines makes them natural adjuvants or preeminent delivery vehicles, useful as tools to guide the design of effective delivery systems that mimic the potent immunostimulatory action of DC; such DC-like delivery systems will be useful in vaccines to induce protective immunity against intracellular pathogens and to unravel the necessary vaccine machinery in terms of Ags, delivery, costimulation, immunomodulation, and homing requirements (50, 51). In fact, DC-based cellular vaccines have shown that, given an effective delivery vehicle, inactivated chlamydial elementary bodies possess sufficient immunogenic epitopes to elicit a protective immunity against Chlamydia. The challenge for vaccinology therefore is to develop a delivery system that mimics the superior immunostimulatory properties of DC to achieve an effective chlamydial vaccine. In particular, the ability of IL-10-deficient APC to rapidly activate a high frequency of protective specific T cells suggested that they could furnish a model APC system for defining the molecular and biochemical determinants for induction of an enhanced T cell response, and the modulation of these factors can lead to more effective vaccines against agents controlled by T cell immunity.

We have used a combination of gene expression analysis of transcriptional activities and proteomics techniques based on 2-DE and MALDI-TOF to investigate the molecular basis for the potency of IL-10-deficient DC as a highly efficient Th1-activating APC system. The objective was to define and identify the determinants of T cell augmentation against intracellular microbial pathogens, which can be applied in vaccine design against specific pathogen, including Chlamydia and mycobacteria. The RT-PCR studies measured the early transcriptional activities of genes encoding molecules that are involved in APC-T cell interaction during Ag presentation and T cell activation. These results revealed that unlike WT DC, Chlamydia-pulsed IL-10KO DCs rapidly acquired markers (CD11c, CD40, and CD83) that are associated with matured DCs, suggesting that early maturation of DCs is an important factor in the enhanced T cell activation. In fact, early maturation of DCs leads to the rapid acquisition of the capacity to express crucial costimulatory molecules such as IL-1β, CD40, and IL-12 that are important for the differentiative activation of T cells along the Th1 pathway. Our results are further corroborated by the fact that under normal physiological conditions of the mammalian host, DC exist as relatively immature mononuclear cells scattered in various mucosal and nonmucosal tissues in the body. On encounter of an antigenic signal (e.g., through the TLR-mediated signals delivered by recognition of pathogen-associated molecular patterns such as CpG, LPS, dsRNA), DC undergo a differentiative maturation from the immature (highly endocytic cells) to mature and activated cells with up-regulated costimulatory molecules that promote enhanced Ag presentation and T cell activation (52). The DC maturational process controls their capacity to process and generate MHC-peptide complexes, their migration toward secondary lymphoid tissues such as lymph nodes, acquisition of costimulatory molecules, and efficient Ag presentation for T cell activation. Thus, whereas Ag handling by fully mature DC can activate T cells and prime immune responses, immature DC may induce tolerance (53) or suboptimal response. In addition, T cells differentiate into specific phenotypes depending on the TCR-epitope combination, the costimulatory stimuli they receive, and the soluble factors present in the inflammatory milieu during activation. Specifically, the presence of IL-12 delivered by APCs is important for signaling via STAT4, which stimulates IFN-γ gene expression and activation-induced differentiation of naive T cells to the Th1 phenotypes (54, 55). In fact, the efficacy of a DC-based cellular vaccine at inducing protective antichlamydial immunity in mice was suppressed by functional IL-12 elimination, which resulted in lack of Th1 activation (56), and the superiority and preferential induction of protective Th1 response by live over inactivated pathogens, including chlamydiae (57–60), was associated with early induction of GM-CSF which promotes DC maturation and production of IL-12 (61). Moreover, the enhanced T cell activation by Ab-mediated delivery of Ag via Fc receptors on DCs (62) was attributed in part to the effective targeting of Ags to critical cytoplasmic compartments for rapid processing, as well as the induction of DC maturation by initiating specific signaling events that may involve the Syk protein tyrosine kinase, leading to DC activation and enhancement of Ag-presenting function (63, 64). Also, ligation of the costimulatory molecule CD40 on DCs cells triggers production of high levels of IL-12 and enhances T cell-stimulatory capacity via APC activation (65). Finally, immunosuppressive agents such as IL-10 and trichosanthin which inhibit surface costimulatory molecule expression (including B7.1) APCs also inhibit T cell activation and IL-2 production (66). Thus, agents that will foster the rapid maturation of DC and support T cell differentiation will be useful vaccine adjuvants for inducing elevated T cell response.

The comparative proteomic results revealed that IL-10-deficient DC expressed specific metabolic properties that differentiated them immunologically from WT DC and therefore explain their potency in Th1 activation. For instance, the costimulatory molecule B7.1 (CD80) and the T cell-attracting chemokine CCL27/CTACK were among the proteins expressed rapidly and earlier in IL-10KO than WT DCs, whereas the anti-IL-12 molecule PPAR-α had limited expression in IL-10KO DC. Cutaneous T cell-attracting chemokine (CTACK; CCL27) is a member of the CC chemokine family and a functional ligand for CCR10. It is important for rapid recruitment of T cells to the vicinity of Ag-bearing DC, which enhances DC-T cell interaction and Ag presentation. Chlamydia-pulsed IL-10KO DC (but not WT DC) also expressed calcium-dependent protein kinase, sodium channel, and NKR-P1A (CD161) that promote IL-1/IL-12 expression. However, they did not express the anti-inflammatory programmed cell death 1 ligand (PD-1), CCR4-NOT complex, and keratinocyte lipid-binding protein. The results provide a molecular basis for the high Th1-activating capacity of IL-10KO APC and could furnish unique immunomodulation targets when designing vaccines against pathogens controlled by T cell immunity. The immunological analysis of an infectious disease system in a compatible and relevant animal model has allowed us to answer fundamental questions in basic immunology and host defense as they relate to the immunoregulatory determinants of T cell immunity. Although possible immunological differences may be encountered in extending murine results to humans, such subtle immunobiological differences can usually be adequately adjusted to maximize the utility and significance of the experimental finding. Finally, a detailed analysis of these molecules involved in the Th1-enhancing ability of IL-10KO APCs and the molecular mechanisms by which they exert their effect could lead to the designing of more effective vaccines against Chlamydia and other microbial pathogens as well as tumors that are controlled primarily by a robust T cell response.

Acknowledgments
We thank Drs. Kathryn L. Kellar and Robert M. Wohlhueter for the technical assistance.

Disclosures
The authors have no financial conflict of interest.
References


