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# Suppression of Endogenous IL-10 Gene Expression in Dendritic Cells Enhances Antigen Presentation for Specific Th1 Induction: Potential for Cellular Vaccine Development<sup>1</sup>

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A new paradigm for designing vaccines against certain microbial pathogens, including *Chlamydia trachomatis*, is based on the induction of local mucosal Th1 response. IL-10 is an anti-inflammatory cytokine that exerts negative immunoregulatory influence on Th1 response. This study investigated whether biochemical modulation of endogenous IL-10 expression at the level of APCs is a practical strategy for enhancing the specific Th1 response against pathogens controlled by Th1 immunity. The results revealed that the high resistance of genetically engineered IL-10<sup>-/-</sup> (IL-10KO) mice to genital chlamydial infection is a function of the predilection of their APCs to rapidly and preferentially activate a high Th1 response. Thus, in microbiological analysis, IL-10KO mice suffered a shorter duration of infection, less microbial burden, and limited ascending infection than immunocompetent wild-type mice. Also, IL-10KO were resistant to reinfection after 8 wk of the primary infection. Cellular and molecular immunologic evaluation indicated that IL-10KO mice induced greater frequency of chlamydial-specific Th1 response following *C. trachomatis* infection. Moreover, IL-10KO APCs or antisense IL-10 oligonucleotide-treated wild-type APCs were potent activators of Th1 response from naive or immune T cells. Furthermore, both Ag-pulsed dendritic cells from IL-10KO mice and IL-10 antisense-treated dendritic cells from wild-type mice were efficient cellular vaccines in adoptive immunotherapeutic vaccination against genital chlamydial infection. These findings may furnish a novel immunotherapeutic strategy for boosting the Th1 response against T cell-controlled pathogens and tumors, using IL-10-deficient APCs as vaccine delivery agents. *The Journal of Immunology*, 2000, 164: 4212–4219.

Genital infection by the obligate intracellular bacterium, *Chlamydia trachomatis*, is the most common sexually transmitted disease in the United States and several other industrialized nations, including the United Kingdom and Germany. A recent report by the World Health Organization revealed that 90 of 500 million annual new global sexually transmitted diseases are attributed to *C. trachomatis* (1, 2). In the United States, four million reported annual cases of genital chlamydial infections cost over \$2 billion (2, 3). Of major pathophysiologic significance is that in women the cervical disease can spread into the upper genital tract, leading to serious complications, such as pelvic inflammatory disease, fallopian tube scarring, ectopic pregnancy, and infertility (4). Moreover, the frequent asymptomatic infections may result in severe irreversible complications that become the first symptoms of an infection. There are concerns that genital chlamydial disease may pose a serious threat to human reproduction, well-being, and healthcare costs. Thus, there are major efforts

to design control and prevention strategies, of which frequent screening programs to ensure early detection and treatment (2, 5) and the administration of an efficacious vaccine have become a priority.

The search for a chlamydial vaccine has led to the development of several animal models for understanding the pathogenesis and immunobiology of the disease. Current research focuses on defining the relevant immune effectors that mediate anti-chlamydial immunity, identify protective Ags that elicit such responses, and design effective methods of vaccine delivery. Recent reports have indicated that rapid, early elicitation and recruitment of certain immune effectors (i.e., dendritic and Th1 cells) into the local genital mucosae are crucial for reducing the intensity of and terminating a cervico-vaginal infection and curbing ascending disease, and therefore are important for preventing major complications of chlamydial infection (6–9). Specifically, it has been established that specific Th1 cells and their notable cytokine, IFN- $\gamma$ , are crucial immune effectors controlling *Chlamydia* in mice, guinea pigs, nonhuman primates, and probably humans (7, 10–16), and these cells are recruited into the genital mucosa during an infection. The induction of mucosal anti-chlamydial Abs, such as secretory IgA and IgG, appears to play an ancillary role in protective immunity (12).

The activation, recruitment, and retention as well as the effector function of Th1 cells against microbial pathogens or tumors involve 1) obligatory intimate interaction with accessory, infected, and noninfected cells via cell surface molecules that include the gene products of the MHC, addressins, coreceptors, and costimulatory and adhesion molecules (17, 18); and 2) cytokine induction and interaction at the afferent and efferent phases of host immune responses. Local mucosal regulation of the expression and activity of these cellular and molecular entities determines the outcome of

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the overall host response as well as the acquisition and maintenance of immunity that ensure vaccine efficacy. In particular, cytokines are important mediators of immunity against microbial pathogens. The pattern of cytokines induced following exposure to specific microbes could determine the outcome of the infection, including the establishment of immunity or the development of disease (19). In this respect it has been shown that for several intracellular pathogens (including *Chlamydia*, *Mycobacteria*, and *Leishmania*), protective immunity requires the induction of Th1 cytokines, notably IFN- $\gamma$ , TNF- $\alpha$ , and IL-2; however, pathology develops if Th2 cytokines (i.e., IL-4, IL-5, and IL-10) are induced (19, 20). The pattern and interaction of Th1 and Th2 cytokines elicited could therefore affect the outcome of an infection. Furthermore, since several pathogens are preferentially susceptible to either Th1 or Th2 response, the identification of immunobiologic factors that selectively regulate Th1 or Th2 response against a given pathogen could aid in skewing protective immune responses to the desired route as part of vaccine design strategy.

IL-10, an anti-Th1 cytokine (21) produced during genital chlamydial infection of experimental animals and humans (22, 23), is potentially an important local factor that could control the Th1 response against *Chlamydia*. Thus, genetic differences in mouse strain susceptibility to *C. trachomatis* or *Coccidioides immitis* was mapped to high IL-10 producers being more susceptible to infections (22, 24). Also, increased IL-10 levels in the endocervical secretion of women with chlamydial infection could predispose to enhanced HIV-1 transmission (23). These observations suggest that the temporary or inadequate immunity commonly induced against *Chlamydia* (12) is at least partially due to the negative immunoregulation of IL-10, which could occur via IL-10-regulated production of relatively low frequency of chlamydial-specific Th1 cells. In such a case, genetic or biologic 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 may mediate long term anti-chlamydial immunity. In this study we revealed that the cellular and molecular immunologic basis for the resistance of IL-10-deficient mice to chlamydial infection is associated with the potency of the APCs at inducing a Th1 response, and that an immunotherapeutic cellular vaccination strategy based on biochemical suppression of IL-10 expression in dendritic cells (DC) <sup>3</sup> might be a practical application of this system in vaccinating against pathogens or tumors that are controlled by Th1 immunity.

## Materials and Methods

### *Chlamydia stocks and Ags*

Stocks of *C. trachomatis* agent of mouse pneumonitis (MoPn) and the human isolate serovar D used to infect mice in vivo were prepared by propagating elementary bodies (EBs) in McCoy cells as previously described (25). Stocks were titrated by infecting McCoy cells with varying dilutions of EBs, and the infectious titer was expressed as inclusion-forming units (IFU) per milliliter. Chlamydial Ag was prepared by growing MoPn in HeLa cells and purifying EBs over renografin gradients, followed by inactivation under UV light for 3 h.

### *Animals, infection, and analysis of the course of the infection*

Female IL-10<sup>-/-</sup> and IL-10<sup>+/+</sup> mice on a C57BL/6J background, 5–8 wk old, were obtained from The Jackson Laboratory (Bar Harbor, ME). All animals were fed food and water ad libitum and maintained in laminar flow racks under pathogen-free conditions of 12 h of light and 12 h of darkness. Mice were infected intravaginally with 10<sup>5</sup> IFU of MoPn/mouse in a vol-

ume of 30  $\mu$ L of PBS while under phenobarbital anesthesia. The course of the infection was monitored by periodic (every 3 days) cervico-vaginal swabbing of individual animals. *Chlamydia* was isolated from the swabs in tissue culture according to standard methods, and inclusions were visualized and enumerated by immunofluorescence (25). The animals were monitored for at least 4–6 wk, a time period that spans the course of MoPn infection in mice (8). Infected KO mice did not show any clinical evidence of overt pathology beside the shedding of chlamydiae in their genital tracts, suggesting that the inoculum was not lethal for the animals. Experiments were repeated to include 10 or 12 animals per experimental group.

### *Assessment of ascending infection*

MoPn was isolated from the upper genital tracts of mice at different times after infection as follows. Mice were infected intravaginally with 10<sup>5</sup> IFU of MoPn/mouse as previously described (25). At the indicated time periods after infection a portion of the reproductive system between the uterus and the ovaries of each mouse was removed and teased with forceps, and tissue homogenates were collected in 1 ml of PBS. *Chlamydia* was isolated from the homogenate in tissue culture according to a standard immunofluorescence staining method (25).

### *Cytokines, mAbs, and other reagents*

ELISA kits for quantitating the amounts of murine cytokines in biological and culture fluids were purchased from BioSource (Camarillo, CA). Chlamydial isolation from cervico-vaginal swabs in tissue culture was assayed by staining infected monolayers of McCoy cells with FITC-labeled, genus-specific anti-chlamydial Abs (Kallestad Diagnostics, Chaska, MN) to detect chlamydial inclusions by direct immunofluorescence (25).

### *FACS analysis*

Single-cell preparations from the indicated organs and tissues of IL-10<sup>-/-</sup> and IL-10<sup>+/+</sup> mice were stained with FITC-labeled mAbs directed against murine CD3, CD4, CD8, CD54 (IL-10), CD71 (transferrin receptor), CD102 (ICAM-2), MadCAM-1, NK, MHC class II, and Mac-1 Ags, according to the manufacturer's protocols (BioSource). Stained cells were analyzed on a FACScan flow cytometer (Becton Dickinson, Sunnyvale, CA) using controls stained with isotype-matched irrelevant Abs. The results are expressed as the proportion (percentage) of positively stained cells in the cell preparation.

### *Measurement of frequency of chlamydial-specific Th1 cells (Th1 frequency) after a primary infection of IL-10KO (IL-10<sup>-/-</sup>) and wild-type (IL-10<sup>+/+</sup>) mice*

A modified procedure of the limiting dilution technique (26) 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 (7). Cultures were established by seeding T cells in a serial doubling dilution into 96-well round-bottom tissue culture plates at 24 wells/dilution. Typically, four and eight dilutions were established for each T cell responder in the range of  $1 \times 10^5$  through  $8 \times 10^2$  cells/well. The T cells were stimulated with APCs from wild-type mice ( $2 \times 10^5$  cells/well) and chlamydial Ag (10  $\mu$ g/ml). Background cultures contained 24 wells with APCs and Ag. After 5 days of incubation the supernatants were assayed for IFN- $\gamma$  by a sensitive ELISA (7). 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. Following 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) (26, 27) that provided both the Th1 frequency and the conformity of the input data with a single-hit Poisson model. Genital tract T cells from naive IL-10<sup>+/+</sup> mice have a Th1 frequency of 15 (range, 9–21).

### *Measurement of efficiency of Ag presentation by splenic APCs from IL-10<sup>-/-</sup> and IL-10<sup>+/+</sup> mice*

The efficiency of Ag presentation by splenic APCs from IL-10KO mice and wild type animals was compared by assessing the ability of gamma-irradiated whole spleen cells to present chlamydial Ags to immune T cells from infected wild-type mice. Spleen cells from *Chlamydia*-infected wild-type mice were enriched for T cells by the nylon wool adherence method (13, 28). Purified splenic cells contained at least 97% CD3<sup>+</sup> cells, as determined by FACS analysis. To assess the Ag-presenting function of X-irradiated splenic cells from either IL-10KOs or control mice,  $2 \times 10^5$  cells were cocultured with  $2 \times 10^5$  nylon wool-purified T cells in the presence or the absence of chlamydial Ag (i.e., UV-inactivated MoPn EBs at 10

<sup>3</sup> Abbreviations used in this paper: DC, dendritic cells; MoPn, mouse pneumonitis; EB, elementary bodies; IFU, inclusion-forming units; IL-10KO, IL-10 knockout; IL-12KO, IL-12 knockout; IL-10<sup>-/-</sup>, IL-10KO phenotype; IL-10<sup>+/+</sup>, wild-type phenotype; IL-12<sup>-/-</sup>, IL-12KO phenotype; oligo, oligonucleotide.

$\mu\text{g/ml}$ ) 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- $\gamma$  content by a quantitative ELISA (Cytoscreen Immunoassay Kit, BioSource) according to the supplier's instructions. The concentration of cytokine in each sample was obtained by extrapolation from a standard calibration curve generated simultaneously. Data were calculated as the mean values ( $\pm$ SD) of triplicate cultures for each experiment. The results were derived from at least three independent experiments.

#### Assessment of the effect of antisense IL-10 oligonucleotide (oligo) treatment of DC on chlamydial Ag presentation and activation of specific Th1 response

DC were isolated from the bone marrow of wild-type (IL-10<sup>+/+</sup>) mice by a standard procedure (29). Briefly, marrow was collected from the femurs and tibia of naive wild-type C57BL/6 mice (6–8 wk old) and refluxed by vigorous pipetting to dissociate the tissue and release the cells. Approximately  $2.0 \times 10^7$  cells were plated in 100-cm<sup>2</sup> dishes in complete RPMI 1640 medium containing 10% FCS, HEPES, glutamine, nonessential amino acids, sodium pyruvate, gentamicin, recombinant murine GM-CSF (10 ng/ml), and IL-4 (5 ng/ml). Following enrichment for DC (29) and washing steps,  $10^5$  cells were plated per well in round-bottom 96-well plates and treated for 24 h with 20  $\mu\text{M}$  of either sense or antisense oligos corresponding to nucleotides 315–333 of the murine IL-10 gene (30, 31) (i.e., sense, 5'-CCAAGCCTTATCGGAAATG-3'; and antisense, 5'-CATTTCGGATAAGGCTTGG-3'). The cells were then used to stimulate splenic T cells from immune mice (at  $10^5$  T cells/well), in the presence or the absence of chlamydial Ag (10  $\mu\text{g/ml}$ ). After 5 days of incubation, the amounts of IFN- $\gamma$  in the culture supernatants were measured by a sensitive sandwich ELISA (7). Concentrations of IFN- $\gamma$  are expressed as the mean (picograms per milliliter) results from three different experiments. Control cultures that contained T cells and APCs but without chlamydial Ag did not show any measurable amount of IFN- $\gamma$ , so the data are not presented. Typically, DC were characterized microscopically as loosely adherent mononuclear cells expressing high levels of MHC class II, CD54, and CD11c but lacking B220 surface Ags.

#### Adoptive immunotherapeutic vaccination with IL-10KO or IL-10 antisense oligo-treated BMDCs

DC were isolated from the bone marrow of IL-10<sup>-/-</sup> and IL-10<sup>+/+</sup> mice as previously described above. Purified DC from IL-10<sup>+/+</sup> mice were treated with either antisense or sense oligos. Then, IL-10KO DC and IL-10 oligo-treated DC were pulsed with *C. trachomatis* serovar D Ag for 12 h. Each DC preparation was adoptively transferred into 4-wk-old female C57BL/6 mice ( $2.5 \times 10^7$  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 and were fed and maintained under a 12-h light, 12-h dark cycle. After 1 wk, the mice were infected intravaginally with  $10^4$  IFU/mouse of live *C. trachomatis* serovar D. The status of the infection was monitored by periodic cervico-vaginal swabbing of individual animal and isolation of chlamydiae in tissue culture (32). Experiments were repeated twice, and there were six to nine mice per group.

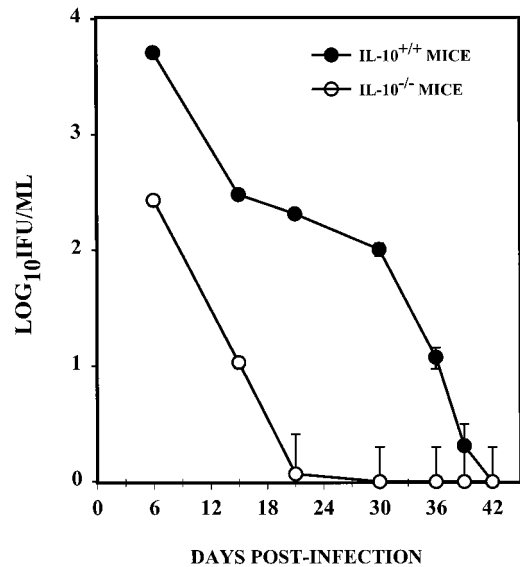
#### Statistical analysis

The levels of IL-2 or IFN- $\gamma$  in samples from different experiments were analyzed and compared by performing a one- or two-tailed *t* test, and the relationship between different experimental groupings was assessed by ANOVA. Minimal statistical significance was judged at  $p < 0.05$ .

## Results

### High resistance of IL-10KO mice to genital chlamydial infection is associated with the predilection to inducing elevated Th1 response

Because of the negative immunologic influence of IL-10 on the Th1 response and the fact that *Chlamydia* immunity in mice is mediated by Th1 cells, we initially tested the hypothesis that genetically engineered IL-10KO (IL-10<sup>-/-</sup>) mice would exhibit greater resistance to genital chlamydial infection than wild-type (IL-10<sup>+/+</sup>) mice. When the course of genital chlamydial infection was compared in IL-10<sup>-/-</sup> and IL-10<sup>+/+</sup> mice by determining the intensity of shedding of chlamydiae into the cervico-vaginal vault, the infection was less severe and of shorter duration in IL-10<sup>-/-</sup> mice (Fig. 1). IL-10<sup>-/-</sup> mice exhibited between 1 and 3 log lower



**FIGURE 1.** Course of genital chlamydial infection in IL-10<sup>-/-</sup> and IL-10<sup>+/+</sup> mice. Female IL-10<sup>-/-</sup> and IL-10<sup>+/+</sup> mice on a C57BL/6 background were obtained from The Jackson Laboratory. Mice were infected intravaginally with  $10^5$  IFU of *C. trachomatis* agent of MoPn. The course of the infection was monitored by periodic (every 3 days) cervico-vaginal swabbing of individual animal. Chlamydiae was isolated from the swabs in tissue culture according to standard methods, and inclusions were visualized and enumerated by immunofluorescence (32). Results are expressed as inclusion-forming units per ml. Experiments were repeated twice, and there were 10 or 12 animals per experimental group.

shedding of chlamydiae and cleared the infection up to 3 wk earlier than IL-10<sup>+/+</sup> mice. Moreover, IL-10<sup>-/-</sup> mice suffered a markedly reduced ascending infection, as determined by the presence of live organisms in the uteri and ovarian regions of the reproductive system. These results suggested that the relevant anti-chlamydial immune effectors are elicited more rapidly and induced in greater amounts in IL-10KO than in wild-type mice. Also, IL-10<sup>-/-</sup> mice are likely to suffer lesser complications associated with ascending chlamydial infection.

It has been established, at least in the murine system, that chlamydial control is mediated by Th1 immunity (12). Therefore, we investigated the hypothesis that a specific anti-chlamydial Th1 response is induced more rapidly, and greater numbers of specific Th1 cells are recruited into the genital mucosa of infected IL-10<sup>-/-</sup> mice compared with those in wild-type IL-10<sup>+/+</sup> mice. Accordingly, limiting dilution analysis (26, 27) was used to compare the kinetics of induction and recruitment of chlamydial-specific Th1 cells into the genital tract of infected IL-10<sup>-/-</sup> and IL-10<sup>+/+</sup> mice. By calculating the frequency of Ag-specific IFN- $\gamma$ -secreting T cells (i.e., Th1 cells) in the genital tract T cell preparations, it was found that IL-10<sup>-/-</sup> mice exhibited a greater and more rapid Th1 response than control mice (Table I). Thus, there were 5- to 50-fold more chlamydial-specific Th1 cells in the genital tracts of IL-10<sup>-/-</sup> mice over 15–85 days of intravaginal infection relative to those in wild-type mice. Moreover, when the mice were challenged after 85 days of the primary infection, IL-10<sup>-/-</sup> mice resisted the productive establishment of a cervico-vaginal infection. In contrast, wild-type mice were productively infected, although they suffered a milder intensity of disease compared with the primary infection. The results suggested that elevated frequency of chlamydial-specific Th1 cells in the IL-10<sup>-/-</sup> mice conferred protective immunity in these mice.

Table I. Frequency of chlamydial-specific Th1 cells (Th1 frequency) after a primary infection of IL-10KO (IL-10<sup>-/-</sup>) and wild-type (IL-10<sup>+/+</sup>) mice

| Day Postinfection | Frequency of Chlamydial-Specific Th1 Cells/106 GT T Cells [Mean value (range)] |                           | p Values |
|-------------------|--|---------------------------|----------|
|                   | IL-10 <sup>-/-</sup> mice  | IL-10 <sup>+/+</sup> mice |          |
| 15                | 465 (310–620)  | 43 (29–57)                | 0.0001   |
| 30                | 689 (492–886)  | 128 (92–165)              | 0.0001   |
| 42                | 1447 (1072–1822)   | 140 (93–187)              | 0.0001   |
| 85                | 1050 (700–1400)  | 21 (14–28)                | 0.0001   |

#### Potency of IL-10KO APCs in stimulating specific Th1 cells

To understand the cellular and molecular immunologic basis for the enhanced Th1 induction in IL-10KO mice, the study focused on the APCs because of their central role in immune initiation and regulation. It was hypothesized that transcriptional control of endogenous expression of IL-10 in APCs would determine whether T cell activation is skewed toward a Th1, Th2, or mixed Th1/Th2 response. To test this hypothesis, the efficiency of APCs from IL-10<sup>-/-</sup> mice at presenting chlamydial Ags to immune T cells from either knockout or wild-type mice was investigated. When T cells from IL-10<sup>-/-</sup> and IL-10<sup>+/+</sup> mice were stimulated with APCs derived from IL-10<sup>-/-</sup> mice, a greater Th1 response was always induced compared with that produced by stimulation with APCs from IL-10<sup>+/+</sup> mice (Fig. 2). The Th1 response was measured by Ag-specific IFN- $\gamma$  secretion, as previously described (7). APCs from IL-10<sup>-/-</sup> mice were ~4 and 15 times more efficient at activating Th1 cells in T cell preparations from IL-10<sup>-/-</sup> and IL-10<sup>+/+</sup> mice, respectively, than APCs derived from IL-10<sup>+/+</sup>

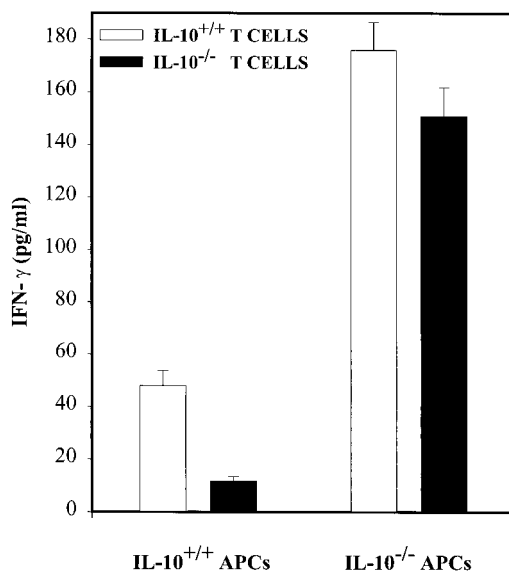


FIGURE 2. Role of APCs in *Chlamydia*-specific Th1 activation. Nylon wool-purified splenic T cells were isolated from MoPn-infected female IL-10<sup>-/-</sup> and IL-10<sup>+/+</sup> mice after 45 days of infection. Purified T cell preparations were stimulated with splenic APCs from either IL-10<sup>-/-</sup> or IL-10<sup>+/+</sup> mice plus chlamydial Ag for 5 days. The amounts of IFN- $\gamma$  in the culture supernatants were measured by a sensitive sandwich ELISA as previously described (7). The concentrations of IFN- $\gamma$  are expressed as the mean (picograms per milliliter) of results from at least three different experiments. Control cultures that contained T cells and APCs but without chlamydial Ag did not show any measurable amount of IFN- $\gamma$ , so the data are not presented in the results shown.

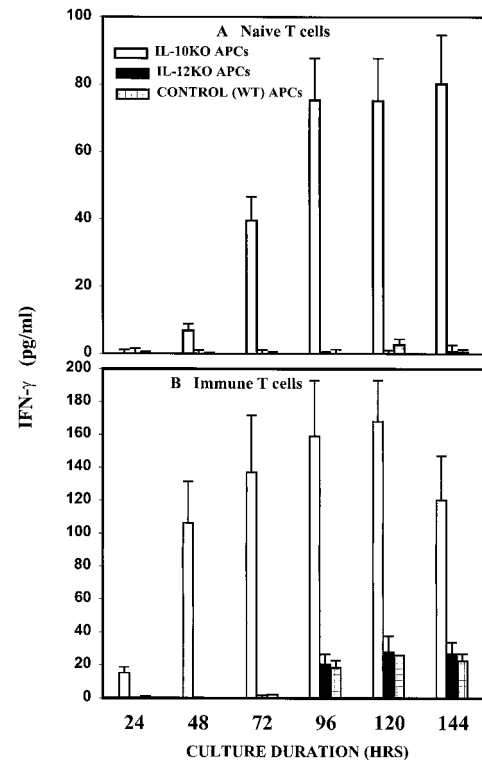
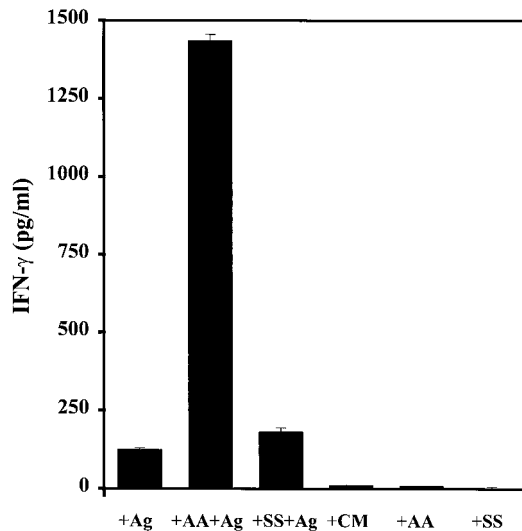


FIGURE 3. Kinetics of Th1 activation by IL-10KO APCs. Nylon wool-purified T cells were isolated from the spleens of naive and genitally *Chlamydia*-infected IL-10<sup>+/+</sup> mice. The T cells (10<sup>5</sup>/well) were stimulated with 10  $\mu$ g/ml of chlamydial Ag and 2  $\times$  10<sup>5</sup> cells/well of APCs from IL-10<sup>-/-</sup>, IL-12<sup>-/-</sup>, or IL-10<sup>+/+</sup> mice. At the indicated time periods after incubation, the amounts of IFN- $\gamma$  in the culture supernatants were measured by ELISA (7). The concentrations of IFN- $\gamma$  are expressed as the mean (picograms per milliliter) of results from at least three different experiments. Control cultures containing T cells and APCs, but no chlamydial Ag, had no measurable amounts of IFN- $\gamma$ , so the data are not presented.

mice. In addition, kinetic studies of the Th1-inducing ability of IL-10KO APCs using either naive or immune T cells from IL-10<sup>+/+</sup> mice showed that IL-10KO APCs were rapid and potent activators of both primary and secondary Th1 responses against chlamydial Ags (Fig. 3). These results indicated that the negative immunoregulatory effect of IL-10 on the Th1 response is exerted at the level of the APCs, a novel finding in the immunobiology of IL-10. Moreover, the enhanced Th1 induction by IL-10KO APCs appears to be an active process, not just a default outcome of lack of IL-10 in the environment. This point is substantiated by the results showing that T cells from IL-10<sup>+/+</sup> mice containing the intact IL-10 gene were stimulated to express an elevated Th1 response by IL-10KO APCs (Fig. 2). Besides, it was interesting that increased levels of IL-2, IL-6, and IL-12 were measured in cultures containing IL-10KO APCs as well, but IL-4 was undetected except in those containing APC from IL-10<sup>+/+</sup> mice (data not shown). Although the role of IL-2 and IL-12 in Th1 development is established (33), the reasons for the concomitant increase in IL-6 in these cultures is unclear. However, a role for IL-6 in host defense against *C. trachomatis* was suggested in a recent analysis of IL-6KO mice (34).

#### Potential application of enhanced Th1 activation by IL-10KO APCs for immunotherapy: potency of IL-10 antisense oligo-treated APCs in stimulating Th1 cells

Genetic or biochemical modulation of endogenous IL-10 expression in APCs for activating a high frequency of specific Th1 cells



**FIGURE 4.** Effect of antisense IL-10 oligo treatment of DC on chlamydial Ag presentation and activation of a specific Th1 response. DC were isolated from the bone marrow of wild-type (IL-10<sup>+/+</sup>) mice using a standard procedure (29). Following enrichment for DC (29), 10<sup>5</sup> cells were plated per well in round-bottom 96-well plates and treated with 20 μM of either sense or antisense oligos corresponding to nucleotides 315–333 of the murine IL-10 gene (30, 31) for 24 h. The cells were then used to stimulate splenic T cells from immune mice (at 10<sup>5</sup> T cells/well), in the presence or the absence of chlamydial Ag (10 μg/ml). After 5 days of incubation, the amounts of IFN-γ in the culture supernatants were measured by a sensitive sandwich ELISA (7). Concentrations of IFN-γ are expressed as the mean (picograms per milliliter) of results from three different experiments. Control cultures that contained T cells and APCs but without chlamydial Ag did not show any measurable amounts of IFN-γ, so the data are not presented. +Ag, cultures containing T cells and chlamydial Ag; +AA + Ag, cultures containing IL-10 antisense oligo-treated DC, and T cells, and Ag; +SS + Ag, cultures containing IL-10 sense oligo-treated DC, T cells, and Ag; CM, cultures containing T cells and DC but no Ag; +AA, cultures containing IL-10 antisense oligo-treated DC and T cells but no Ag; +SS, cultures containing IL-10 sense oligo-treated DC and T cells but no Ag.

holds promise for ex vivo manipulation of APCs, such as DC, in adoptive immunotherapy to vaccinate against chlamydial infection and other pathogens controlled by the Th1 response. We investigated whether biochemical modulation of endogenous IL-10 expression in normal DC using an antisense oligo approach would foster Ag presentation for enhanced specific Th1 activation. DC were isolated from the bone marrow of wild-type mice and characterized as loosely adherent mononuclear cells, expressing high levels of MHC class II, CD54, and CD11c, but lacking B220 surface Ags (29). Purified DC were treated with 20 μM of either the antisense or the sense oligo of the murine IL-10 gene (30, 31) and used to stimulate splenic T cells from immune mice. The results presented in Fig. 4 reveal that antisense treatment of DC boosted their efficiency at activating a specific Th1 response against chlamydial Ags. There was at least an 11-fold increase in Th1 induction by antisense-treated DC over untreated DC. FACS analysis showed that antisense oligo-treated DC expressed decreased levels of FcγII/III receptors (CD16/CD32;  $p > 0.0001$ ), but the levels of CD54, CD11b, CD11c, and MHC class II Ags were essentially unchanged. These results favor the likelihood that antisense IL-10 oligo treatment of APCs is a viable ex vivo approach to biochemical modulation of endogenous IL-10 expression and enhancement of Th1 activation. However, the ultimate goal of this phenomenon is to determine whether IL-10-suppressed APCs can function as

**Table II.** Protection of mice from chlamydial genital infection by adoptive immunotherapeutic vaccination with IL-10KO or IL-10 antisense oligo-treated DC

| Day Postinfection | Incidence of Disease in Mice (no. infected/no. without infection) |                  |                                      |  |                          |
|-------------------|---|------------------|--------------------------------------|--|--------------------------|
|                   | DC only, group 1  | DC + Ag, group 2 | IL-10 sense-treated DC + Ag, group 3 | IL-10 antisense-treated DC + Ag, group 4 | IL-10KO DC + Ag, group 5 |
| 6                 | 6/6   | 6/6              | 6/6                                  | 1/9                                      | 1/9                      |
| 15                | 6/6   | 6/6              | 6/6                                  | 1/9                                      | 0/9                      |
| 21                | 6/6   | 5/6              | 4/6                                  | 0/9                                      | 0/9                      |
| 30                | 5/6   | 3/6              | 1/6                                  | 0/9                                      | 0/9                      |
| 42                | 0/6   | 0/6              | 0/6                                  | 0/9                                      | 0/9                      |

cellular vaccines, capable of processing, selecting, and delivering appropriate immunogenic epitopes to the host immune system, for activating a high frequency of specific Th1 response in vivo and establishment of protective immunity against an infection.

#### *Immunotherapeutic vaccination using IL-10KO DC or IL-10 antisense oligo-treated DC for inducing protective immunity against genital chlamydial infection*

In exploring novel vaccine-designing strategies, it has been shown that an immunotherapeutic cellular vaccination approach using DC as potent inducers of a T cell response is a viable strategy for inducing protective immunity against the complications of genital chlamydial infection (29, 35). Therefore, using the adoptive immunotherapeutic vaccination technique, we evaluated the ability of a cellular vaccine, comprising chlamydial-pulsed IL-10KO or IL-10-suppressed DC, to confer protective immunity against genital infection by a human serovar of *C. trachomatis*. The choice of a human serovar for this study include the need to show that the proposed regimen is effective against the human pathogen, which is the main focus of present vaccine efforts, and that the IL-10 effect is not unique to the murine system. The hypothesis tested is that the enhanced Th1-inducing capacity of IL-10KO DC should activate a high frequency of chlamydial-specific Th1 cells that would confer protective immunity against an infection. IL-10KO DC isolated from IL-10<sup>-/-</sup> mice were pulsed with UV-inactivated *C. trachomatis* serovar D EBs for 12 h, while wild-type DC from IL-10<sup>+/+</sup> mice were pretreated with either the antisense or sense oligo of the murine IL-10 gene (30, 31) and then pulsed with UV-inactivated *C. trachomatis* serovar D EBs for the final 12 h of a 40-h oligo treatment. Both DC preparations were adoptively transferred into naive female mice, and after 1 wk the mice were challenged intravaginally with live *C. trachomatis* serovar D. Table II shows the results of the adoptive transfer studies to evaluate the ability of IL-10 suppressed DC to form the basis of a cellular vaccine for inducing a specific Th1 response that protects against *Chlamydia*. All recipients of DC only (group 1), Ag-pulsed DC (group 2), and Ag-pulsed, IL-10 sense oligo-treated DC (group 3) were productively infected during the first 2 wk of challenge. However, only one of nine recipients of Ag-pulsed IL-10KO DC (group 5) and Ag-pulsed, IL-10 antisense oligo-treated DC (group 4) showed evidence of a productive infection during the same period after challenge infection. In fact, all nine recipients of Ag-pulsed IL-10KO DC (group 5) had resolved the infection by the second week of challenge. In terms of chlamydial titers calculated during the first week (day 6) of challenge, group 1 mice had an average titer of  $6.4 \times 10^3$  IFU/ml, group 2 mice had an average titer of  $2.1 \times 10^3$  IFU/ml, group 4 mice had an average titer of 92.12 IFU/ml, and group 5 mice had an average titer of 20.47

IFU/ml. The results indicated that the exquisite ability of IL-10KO DC to rapidly induce a high frequency of specific Th1 response may underlie the therapeutic potency of the cells in vaccinating against genital chlamydial infection.

## Discussion

The design of an immunization regimen capable of inducing a mucosal Th1 response is the current goal for a human vaccine to control the severe complications of genital infection by *C. trachomatis* (10, 12, 36, 37). Despite considerable effort and clinical and experimental evidence suggesting that at least partial protective immunity is feasible in humans (12), the development of reliable chlamydial vaccines using conventional immunization strategies has proven elusive. However, in addition to inducing a mucosal Th1 response, a potential anti-chlamydial vaccine should have long term protective benefits. To define specific conditions that will influence the efficacy and long term benefits of an anti-chlamydial vaccine, our research has focused on identifying immunologic, endocrine, and local mucosal factors that regulate the Th1 response against *C. trachomatis*. IL-10 is an anti-Th1 cytokine (21) produced during genital chlamydial infection of experimental animals and humans (22, 23). Genetic differences in mouse strain susceptibility to *C. trachomatis* or *Coccidioides immitis* was mapped to high IL-10 producers being more susceptible to infections (22, 24). Also, increased IL-10 levels in the endocervical secretion of women with chlamydial infection could predispose infected individuals to enhanced HIV-1 transmission (23). Taken together, it is conceivable that the temporary or inadequate immunity commonly induced against *Chlamydia* (12) is at least partially due to the negative immunoregulation of IL-10, causing the production of relatively low frequency of chlamydial-specific Th1 cells. In such a case, genetic or biologic suppression of IL-10 activity or expression during immune elicitation against *Chlamydia* should lead to the induction of high levels of a specific Th1 response that may mediate long term anti-chlamydial immunity.

To investigate the potential application of a cellular vaccine system based on the suppression of endogenously expressed IL-10 to facilitate specific Th1 induction, we initially analyzed genital chlamydial infection in genetically engineered IL-10 knockout (IL-10<sup>-/-</sup>) and control wild-type (IL-10<sup>+/+</sup>) mice. The course of genital chlamydial infection was shorter and the disease was less severe in IL-10<sup>-/-</sup> than IL-10<sup>+/+</sup> mice. Moreover, protective, specific anti-chlamydial Th1 cells are elicited more rapidly and induced in greater amounts in IL-10KO mice than in wild-type mice. Thus, IL-10<sup>-/-</sup> mice suffered fewer complications associated with ascending chlamydial infection. Protective immunity induced in IL-10KO was long lasting, because challenged IL-10<sup>-/-</sup> mice resisted the productive establishment of a cervico-vaginal infection. The elevated frequency of chlamydial-specific Th1 cells in the IL-10<sup>-/-</sup> mice may be responsible for the protective immunity displayed by these mice. These findings corroborate previous reports that the resistance of experimental animals to genital reinfection by *Chlamydia* is a function of the intensity of chlamydial-specific T cells in the genital mucosa (28). The resistance of IL-10<sup>-/-</sup> mice to experimental chlamydial lung infection was associated with an increased Th1 response (38), and elevated levels of IL-10 in the endocervical secretions of women with genital chlamydial infection could predispose the women to HIV-1 transmission (23). Therefore, a vaccine delivery system that includes a means of suppressing IL-10 would foster the induction of a high frequency of specific Th1 cells and probably lead to the establishment of long term immunity against *Chlamydia* and possibly other pathogens controlled by the Th1 response.

An understanding of the cellular and molecular immunologic basis of Th1 augmentation against *Chlamydia* in the IL-10-deficient mouse system may furnish information on how biochemical modulation of IL-10 expression can be exploited in vaccine design against *C. trachomatis* and other pathogens controlled by Th1 immunity. Since complete abrogation of IL-10 from the host has obvious adverse immunoregulatory and biologic consequences, the study focused on the APCs because of their central role in immune initiation and regulation. These studies have revealed that the negative immunoregulatory effect of IL-10 on the Th1 response is exerted at the level of the APCs, a novel finding in the immunobiology of IL-10. Moreover, the enhanced Th1 induction by IL-10KO APCs appears to be an active process, not just a default outcome of a lack of IL-10 in the environment, a finding that would underscore the basis for the ineffectiveness of anti-IL-10 Abs to enhance Th1 induction via binding and neutralization of secreted IL-10. Although previous reports demonstrated that exogenous treatment with IL-10 could suppress T cell activation via action on the APCs (39, 40), the present studies have shown, for the first time, that endogenous IL-10 production by the APCs is a crucial regulatory step in Th1 activation. The observed increased levels of IL-2, IL-6, and IL-12 in cultures containing IL-10KO APCs but undetectable levels of IL-4 posed a challenging explanation. Although the role of IL-2 and IL-12 in Th1 development is established (33), the concomitant increase in IL-6 in these cultures is unclear. However, a role for IL-6 in host defense against *C. trachomatis* was suggested in recent analysis of IL-6KO mice (34). Furthermore, it is important to mention that the Th1 response measured in these studies would include cellular immune responses mediated principally by CD4<sup>+</sup> Th1 cells, but may also include IFN- $\gamma$ -secreting CD8<sup>+</sup> T cells.

These studies indicated that genetic or biochemical modulation of endogenous IL-10 gene expression in APCs for activating a high frequency of specific Th1 cells holds promise for ex vivo manipulation of APCs, such as DC, in adoptive immunotherapy to vaccinate against chlamydial infection and other pathogens controlled by the Th1 response. The findings extend previous reports by Caldwell and co-workers (29, 35) that chlamydia-pulsed DC are potent inducers of cell-mediated immunity and are capable of vaccinating against genital chlamydial infection in mice. DC are prime candidates for immunotherapeutic vaccination against several pathogens and tumors (41–46) because of their proclivity for activating specific T cells against a number of Ags (47–51), including chlamydial Ags (29, 52). The potency of DC as highly efficient APCs is due in part to their high costimulatory ability associated with an elevated density expression of costimulators such as IL-1, LFA-3, and B7 molecules (41, 53, 54). Beside a decrease in surface expression of Fc $\gamma$ R molecules, the high potency of IL-10 antisense oligo-treated DC at activating the Th1 response could not be explained by an immediate effect on the common costimulatory molecules on the APCs. FcRs are important mediators of the effector functions of Abs in humoral immunity associated with the Th2 response. Suppression of FcR expression on APCs could be an indicator of an immune response being skewed to inducing a predominantly Th1 response. Furthermore, it is possible that the effect of antisense treatment was manifested at a later time when the APCs are exposed to T cells in the presence of Ag. The phenomenal efficacy of these cellular vaccines makes them appropriate candidates for inducing protective immunity against tumors and pathogens controlled by Th1 immunity, including *Chlamydia*, HIV, *Mycobacteria*, as well as certain protozoan parasites. Interestingly, recent clinical studies in humans suggested that increased IL-10 levels in the endocervical secretion of women with chlamydial infection could predispose to enhanced HIV-1 transmission

(23), because the condition limits the induction of a Th1 response, which is critical for HIV-1 control.

IL-10-suppressed DC-based cellular vaccines have unique advantages that could facilitate rapid extension of the experimental technology to clinical applications. First, the remarkable ability of DC to process a whole Ag or components of an Ag and select the appropriate immunodominant epitope(s) for presentation to and activation of specific Th1 cells is a property that may obviate the current search for protective Ags and laborious mapping of immunogenic epitopes. Second, the potential for clinical application of DC in immunotherapies has resulted in the establishment of the technology and protocols for efficient ex vivo propagation of DC from peripheral blood cells of humans (41, 42, 45, 46). However, to be of widespread attraction and application, IL-10 suppressed DC-based cellular vaccines should elicit long term protective immunity, and possibly induce cross-protection from other *C. trachomatis* serovars or species. The potential to induce long term protective immunity is predictable from results showing a high frequency of chlamydial-specific Th1 cells (Table I) and resistance of IL-10<sup>-/-</sup> mice to reinfection 85 days after the primary infection. Furthermore, in its ideal form, adoptive immunotherapeutic cellular vaccination requires an autologous or syngeneic system to avoid alloreactivity associated with foreign transplantation Ags on the transferred cells. However, as in transplantation therapy, there appears to be numerous severe disease conditions (infectious and noninfectious) that require up-regulation of the Th1 response, which warrants the application of an IL-10-suppressed DC-based vaccination strategy for clinical use.

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