RESEARCH ARTICLE

Plasmacytoid dendritic cells modulate nonprotective T-cell responses to genital infection by *Chlamydia muridarum*

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Abstract

Given their immune-modulating capacity, regulatory T cells (Treg) cells may be important players in the induction of the protective T-cell response (Th1) to genital chlamydial infection. Recent work has demonstrated that plasmacytoid dendritic cells (pDC) respond to genital chlamydial infection, and that pDC may be uniquely positioned for the induction of Treg cells during this infection. Here, we present the first data demonstrating that Treg influx into the draining lymph node and the site of infection during genital chlamydial infection. We found that pDC depletion altered the numbers of Treg and nonprotective inflammatory cells [interferonγ-(IFNγ)-producing CD8+ T and IFNγ-producing natural killer T cells] in the spleens of mice genitally infected with *Chlamydia muridarum*. Furthermore, pDC depletion did not alter Th1 cell numbers, indicating that pDC modulate cells that could inhibit and promote nonprotective inflammation during genital chlamydial infection. Finally, we demonstrate that depletion of pDC results in less severe dilation and collagen deposition in the oviduct following resolution of infection, implicating pDC activity in the formation of sequelae following genital *C. muridarum* infection.

Introduction

Genital infection by *Chlamydia trachomatis* is the most prevalent bacterial sexually transmitted disease worldwide (WHO, 2004). Sequelae of these infections include the related pathologies of pelvic inflammatory disease, ectopic pregnancy, and infertility. As a result of the prevalence and sequelae of *C. trachomatis* infection, recent efforts have focused on the development of a vaccine to curtail the spread of this pathogen (Brunham & Rey-Ladino, 2005). These efforts have yet to result in an efficacious vaccine due, in part, to the lack of understanding of the immunological factors required to elicit a protective and nonpathological response to genital infection by *C. trachomatis*. T cells are central in the eradication of chlamydial infection, but are also implicated in exacerbating the sequelae associated with genital chlamydial infection (Brunham & Rekart, 2008). Therefore, recent efforts to develop a vaccine against *C. trachomatis* genital infection have focused on the induction of T cells that do not promote immune-mediated pathology. As dendritic cells (DC) are central in the induction of T-cell responses, recent work has focused on the involvement of DC during genital chlamydial infection (Rey-Ladino *et al.*, 2005, 2007; Moniz *et al.*, 2009).

The capacity of DC to elicit varying immune responses is due, in part, to the existence of multiple DC subsets. The examination of DC subsets responding to genital chlamydial infection revealed that two DC subsets respond differentially to this infection. These include a CD11b+ conventional DC (cDC) and a plasmacytoid DC (pDC) subset (Moniz *et al.*, 2009). The cDC subset seems to be most well poised for the induction of a protective Th1 response, whereas pDC do not appear to be capable of directly interacting with T cells and inducing this response during chlamydial genital infection (Moniz *et al.*, 2009). Alternatively, pDC activity during genital chlamydial infection indicates that pDC may be

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poised to promote tolerance during chlamydial infection through the induction of regulatory T cells (Treg). The capacity of pDC to induce Treg has been shown in several disease models (de Heer *et al.*, 2004; Ochando *et al.*, 2006; Goubier *et al.*, 2008), and has particular significance for chlamydial infection. In particular, the immunomodulatory potential of Treg may prove to be significant, given the implication of immune-mediated pathology during genital chlamydial infection. In this work, we examined the role that pDC may play in regulating various T-cell subsets in the hope of providing further insight into the formation of protective and pathological responses during genital *C. trachomatis* infection.

Materials and methods

Chlamydia muridarum propagation and infection

Chlamydia muridarum (Nigg strain) was grown, purified, and titrated in McCoy cells as described previously (Maxion *et al.*, 2004). Elementary bodies and reticulate bodies were isolated from McCoy cells and frozen in sucrose-phosphate-buffered saline (SPS) at $-80\,^{\circ}$C until use. Female BALB/c mice, aged 5–6 weeks, were treated with progesterone [Depo-Provera, 2.5 mg in 100 μL sterile phosphate-buffered saline] 1 week before infection to synchronize mice hormonally. Following anesthetization, mice were inoculated with 1×10^5 infection-forming units (IFU) intravaginally. For determination of bacterial load, mice were swabbed vaginally every 3 days after infection. Swabs were placed in 200 μL SPS and frozen at $-80\,^{\circ}$C before determination of bacterial load. Bacterial load (IFU) was determined in McCoy cells, as described previously (Maxion *et al.*, 2004). Mice were housed and treated in accordance with the American Association of Accreditation of Laboratory Animal Care guidelines. Experimental procedures were approved by the UCLA Institutional Animal Care and Use Committee.

Isolation and analysis of T-cell subsets

T cells were isolated from individual mice on the indicated day after infection. For analysis of T cells from the genital tract (GT), tissues were excised from individual mice, diced, and cultured for 1 h at 37 °C in the presence of collagenase (1 mg mL^{-1}, Type I, Sigma) and DNAse (0.4 mg mL^{-1}, Sigma) in Hank's balanced salt solution (HBS) with Ca^{++} and Mg^{2+}. The spleens and iliac nodes (ILN) were diced and kept on ice. Single-cell suspensions were obtained by mincing tissue through a 70-μm filter (Falcon) in HBS without Ca^{2+} and Mg^{2+}, 5 mM EDTA, and 0.5% BSA. Cells were washed twice and maintained in a solution containing 5 mM EDTA to minimize cell adhesion. Cell suspensions from the GT and spleen were applied to lympholyte M gradients (Cedarlane Labs) and spun at 1200 ***g*** for 20 min at room temperature. Low-density cells at the interface were collected by aspiration and washed twice.

Identification of T-cell subsets was performed by fluorescence-activated cell sorting (FACS) as described previously for DC subsets (Moniz *et al.*, 2009), with some modification. Briefly, the lymphocytes obtained above were cultured at a concentration of 2×10^6 cells mL^{-1} in complete medium (RPMI 1640 with 10 mM HEPES, 10 mM sodium pyruvate, 2 mM L-glutamine, 10 mM nonessential amino acids, 10% v/v fetal calf serum (FCS), and 100 μg mL^{-1} penicillin and streptomycin) in the presence of brefeldin A (10 μg mL^{-1}), phorbol myristate acetate (5 ng mL^{-1}), and ionomycin (500 ng mL^{-1}, all from Sigma) at 37 °C in a tissue culture incubator. After 4 h, DNAse was added to a final concentration of 200 μg mL^{-1} and incubated at room temperature for 15 min on an orbital rotator. The suspension was washed and then stained for FACS analysis. T-cell subsets were defined as live CD3ε+ (145-2C11) CD4+ (RM4-5) cells expressing either IFNγ (Th1, XMG1.2) or FoxP3 (Treg, FJK-16s). Analysis of Th1 and Treg cells was performed simultaneously. Evaluation of additional T-cell subsets was performed separately by analysis of live CD3ε+ CD8α+ (CD8+ T cells, 53-6.7) cells or CD3ε+ CD49b+ [natural killer (NK) T cells, DX5] cells. FACS acquisition was performed on a FACSCaliber (Becton Dickinson) at the UCLA Jonsson Comprehensive Cancer Center and Center for AIDS Research Flow Cytometry Core Facility. FACS analysis was performed using FCS Express (DeNovo Software). For each indicated time point, 10 mice were examined individually from two independent experiments.

pDC depletion

Mice were treated as described above. At 1 day before infection, mice were injected with 500 μg anti-BST2 (mAb 927) antibody or 500 μg isotype control (IgG2b, Biolegend) to deplete pDC (de Heer *et al.*, 2004; Blasius *et al.*, 2006). Subsequent injections were performed every 3 days thereafter until day 8 postinfection, where mice were either sacrificed on day 9 postinfection to examine pDC depletion levels or at 7 weeks postinfection to examine the course and sequelae of infection. pDC depletion efficiency was determined by analysis of pDC numbers in the ILN, GT, and spleen of individual mice by FACS as described previously (Moniz *et al.*, 2009). Briefly, this examination consisted of isolation of DC using density centrifugation (15% metrizamide) and subsequent FACS analysis of the low-density fraction. pDC were examined by gating on CD11c+ CD3− CD19− CD45R(B220)+ cells. Percent depletion of pDC was determined by comparison of total pDC per tissue vs. isotype control injections.

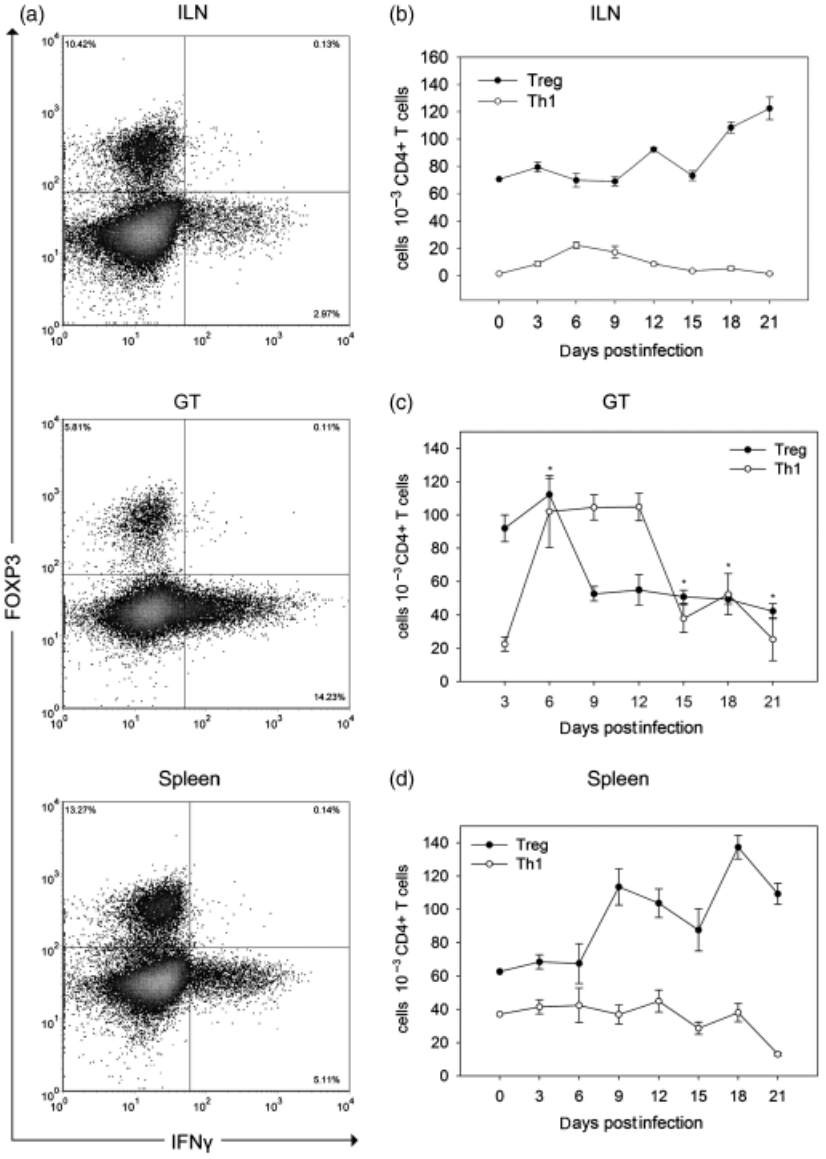

Fig. 1. Treg and Th1 cells show differential responses to genital infection by *Chlamydia muridarum*. (a) Representative FACS plots of T-cell subsets from the indicated tissue, gated on live CD3+ CD4+ cells on day 9 postinfection. (b–d) Kinetic analysis of Treg (CD3+ CD4+ FoxP3+) and Th1 (CD3+ CD4+ IFNγ+) cells in the indicated tissue. Points represent the mean number of cells in individual mice ± SEM with $n = 10$ for each time point. The comparison between the T-cell subset numbers revealed a significant difference in the ILN and spleen, but not the GT, by two-way ANOVA, $P < 0.001$. Likewise, *post hoc t*-test analysis showed differences between Treg and Th1 numbers at all time points analyzed for the ILN and spleen. The number of Treg and Th1 cells at individual time points was analyzed using a *t*-test. *no significant difference on that day using a *t*-test in the GT. All other values in the GT were significantly different using a *t*-test. The number of Tregs reduced over the course of infection by one-way ANOVA, $P < 0.001$, followed by Dunn's *post hoc* test $P < 0.05$.

Histological analysis

Tissue was paraffin-embedded and sectioned, and evaluated by an experienced veterinary research pathologist using blinded samples. Sections stained with hematoxylin and eosin were evaluated for oviduct dilatation using an established semi-quantitative scoring system from 1 to 5 (1+ = $\leq$ 1.0; 2+ = $\leq$ 2.0; 3+ = $\leq$ 3.0; 4+ = $\leq$ 4.0; and 5+ = $>$ 4.0). Sections stained with trichrome were evaluated for fibrosis using digital color microscopy on an Aperio instrument (Vista, CA) using semi-quantitative scoring from 1 to 4 (1+ = $<$ 25% light blue oviducts; 2+ = $>$ 25% light blue oviducts; 3+ = $<$ 25% dark blue oviducts; and 4+ = $>$ 25% dark blue oviducts) (Dahab *et al.*, 2004; Krajewska *et al.*, 2009; Shimazaki *et al.*, 2009).

Statistical analysis

Kinetic changes in the frequency of Treg or Th1 cells were determined using two-way ANOVA with $n = 10$ (Fig. 1b–d). Comparison of the numbers of Treg or Th1 cell numbers throughout the course of infection in the ILN, GT, and spleen was performed using two-way ANOVA and Dunn's *post hoc* test. Statistical comparisons of Treg vs. Th1 cells on individual days were performed using a *t*-test (Fig. 1b–d). Bacterial loads *in vivo* were measured for mice depleted of pDC or treated with control antibody, and the mean IFU mL^{-1} for each treatment was compared using two-way repeated measures ANOVA, with $n = 12$ (Fig. 2b). The effect of pDC depletion of the ratio of the number of Treg and Th1 cells was performed using a *t*-test on day 9 postinfection,

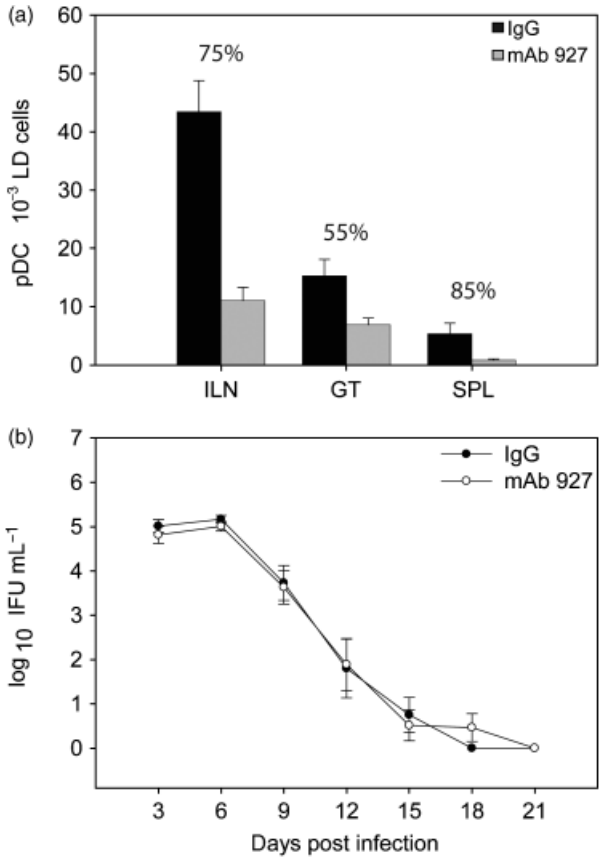

Fig. 2. pDC depletion does not alter the course of infection. (a) Mean numbers of pDC in the indicated tissue from a low-density enrichment fraction. Percentages indicate the mean percent depletion of pDC in mice treated with the mAb 927 antibody vs. mice treated with a nonspecific antibody (IgG) ± SEM. Data represent the mean depletion of two independent experiments on day 9 postinfection. (b) Bacterial load of mice treated as in (a) and described in Materials and methods. Error bars indicate SEM, with no significant difference found between treated groups by two-way repeated measures ANOVA.

with $n = 10$ (Fig. 3a). Oviduct histology scoring of individual control or pDC-depleted mice was compared using the nonparametric Mann–Whitney rank sum test, with $n = 12$, and representing two independent experiments (Fig. 4b–c). The above statistical tests were suggested by and performed using SIGMASTAT software based on the distribution of the data and sample size (Jandel Scientific, San Rafael, CA). Groups were considered statistically different at $P < 0.05$.

Results

Th1 and Treg cells respond differentially following genital chlamydial infection

The role of Treg during genital chlamydial infection is not well defined. Given the immune-modulating potential of these cells, Treg activity would seem to be immediately relevant to the formation of the protective, CD4+ IFNγ-producing T-cell response (Th1). Therefore, we examined the induction of Treg in relation to Th1 cells to establish the balance between these T-cell subsets during the course of chlamydial infection. To this end, we utilized intracellular FACS analysis to characterize CD3+ CD4+ lymphocytes from infected tissues of individual mice for Treg (FoxP3) and Th1 (IFNγ) cells (Fig. 1). Our examination included the entire course of infection to approximately 1 week after bacteria were undetectable by vaginal swabs. In the draining ILN where T-cell responses are initiated, Treg were dominant throughout the course of infection and continued to increase in number after the resolution of infection (Fig. 1b). Alternatively, Th1 cells increased in the ILN only during time points in which bacterial loads were detectable by vaginal swabs, and returned to levels comparable to naïve mice following clearance of infection. A similar distribution of Treg and Th1 cells was found in the spleen, although increases in Th1 cells were less than those observed in the ILN (Fig. 1d). Together, these data demonstrate that the number of Treg (FoxP3+) increase in peripheral lymphoid tissue as a result of genital infection, and remain at elevated numbers in these tissues beyond clearance of infection. Alternatively, Th1 numbers in these tissues closely follow the course of infection.

In the GT tissue, the ratio of Treg to Th1 cells differed from that seen in the secondary lymphoid organs. At early time points following infection, we found Treg to be present in the GT in higher numbers than Th1 (Fig. 1c). Coincident with an increase in Th1 numbers in the ILN (Fig. 1b), these cells significantly outnumbered Treg in the GT and remained in higher numbers than Treg throughout the course of infection. Following clearance of infection (as measured by the undetectable bacterial load, data not shown, Th1 cell numbers reduced in the GT. Surprisingly, Treg numbers also diminished during the course of infection compared with preinfected levels, which suggests that Treg cells were either leaving GT possibly to secondary lymphoid tissues or dying. Thus, similar to the ILN and spleen, the presence of Th1 cells in the GT closely mimics the course of infection, indicating that Th1 retention in the GT is dependent on a productive infection. Alternatively, Treg numbers reduce early and then remain constant throughout and beyond the course of infection.

pDC depletion does not alter the course of infection or Th1 numbers, but does alter Treg numbers in the spleen

Based on our previous work and that of others, a possible role for pDC during chlamydial infection may be to regulate Treg cell numbers. To examine this possibility, we devised a

pDC depletion strategy starting before infection and continuing through day 10 after infection. We depleted pDC using a monoclonal antibody (mAb 927) against the BST2 antigen expressed on pDC (see Materials and methods). The antibody has been characterized for *in vivo* depletion (Blasius *et al.*, 2006). This strategy was used to deplete pDC throughout early infection when DC and T cells interact to generate T-cell subsets as shown in Fig. 1b and previous studies (Moniz *et al.*, 2009). We found this method to be effective in depleting pDC in all the tissues examined, with the most marked depletion of 85% occurring in the spleen (Fig. 2a). Vaginal swabs revealed that in mice depleted of pDC, there was no change in the bacterial load at any time point during the course of infection when compared with mice treated with isotype control antibody (Fig. 2b). This indicates that although the methodology was sufficient in depleting pDC, there was no effect of this depletion on the course of infection.

Although this level of pDC depletion *in vivo* did not alter the overall chlamydial burden in the GT, we reasoned that fewer pDC may alter a non-Th1 subset. We determined the ratio of the number of Tregs (CD3+ CD4+ FoxP3+) in relation to Th1 cells and (CD3+ CD4+ IFNγ+) cells in the ILN, GT, and spleens of individual mice on day 9 postinfection of control and pDC-depleted mice. In the ILN and GT, we found no significant difference in the ratio of the numbers of Treg : Th1 cells (Fig. 3a). This is consistent with the observation that mice depleted of pDC showed no change in the course of infection and indicates that pDC do not directly modulate Th1 cells as suggested by our previous study (Moniz *et al.*, 2009). However, our analysis found that pDC depletion resulted in a significant decrease in the ratio of the number of Treg to Th1 cells in only the spleen (Fig. 3a). Examination of the individual number of Tregs and Th1 cells showed that the number of Tregs significantly decreased while that of Th1 cells remained similar. This indicates that during genital chlamydial infection, pDC modulate the numbers of Treg, but not Th1 cells in the spleen.

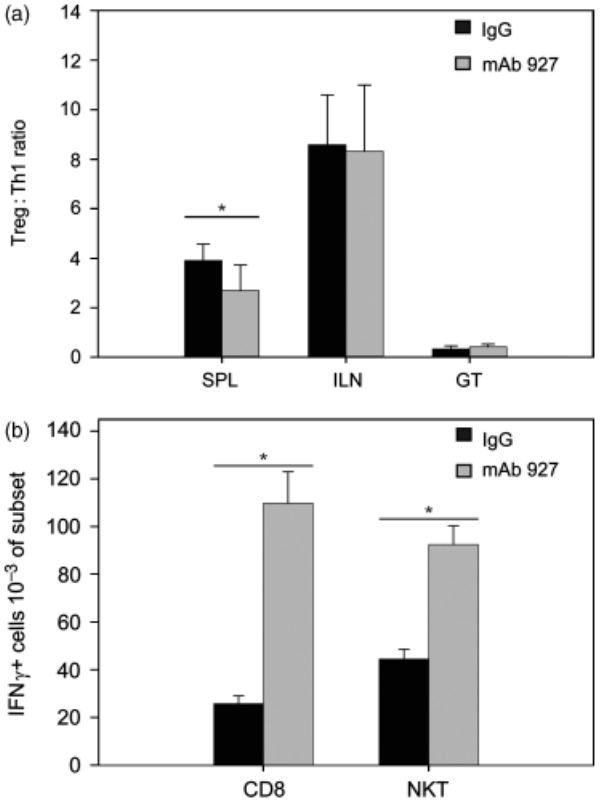

Fig. 3. pDC depletion affects distinct T-cell populations in the spleen. T-cell subsets were isolated from infected mice depleted of pDC (mAb 927) or treated with control antibody (IgG) and analyzed as described in Materials and methods. Data represent the mean number of cells in individual mice on day 9 postinfection, with $n = 10$ for (a) (Treg) and (b) (Th1) and $n = 5$ for (c) (CD8 and NKT) ± SEM. $^{**}P < 0.05$ by a paired *t*-test. $^{*}P < 0.001$ by a *t*-test.

pDC depletion alters the numbers of nonprotective T cells

As pDC may also affect the numbers and activity of T cells other than Treg, we next examined the effect of pDC depletion on T-cell subsets shown to be activated during chlamydial infection. Our efforts focused on T cells that have been shown or have the capacity to produce IFNγ during chlamydial infection, CD8+ T cells and NKT cells, respectively. In the ILN and GT, we found no difference in the numbers of CD8+ IFNγ-producing T cells of mice depleted of pDC vs. mice treated with the control antibody (data not shown). In the spleen, however, there were significantly elevated numbers of CD8+ IFNγ-producing T cells in mice depleted of pDC vs. control mice (Fig. 3b). Likewise, NKT cells were not detectable in the ILN or the GT on day 9 postinfection (data not shown), but were detectable in the spleen, where depletion of pDC resulted in a significant increase in the number of IFNγ-producing NKT cells (Fig. 3b). These data demonstrate that pDC depletion increases the number of IFNγ-producing CD8+ T cells and NKT cells in the spleen during genital chlamydial infection.

pDC depletion results in decreased sequelae of genital chlamydial infection

pDC have been shown to have a broad tolerogenic potential that includes limiting inflammation. As prolonged inflammation is implicated in the sequelae of genital chlamydial infection (Brunham & Rekart, 2008), we examined the formation of sequelae in mice depleted of pDC. To this end, we examined the GT of mice for prototypical signs of sequelae formation, oviduct dilation and collagen deposition (Cohen & Brunham, 1999). Seven weeks after

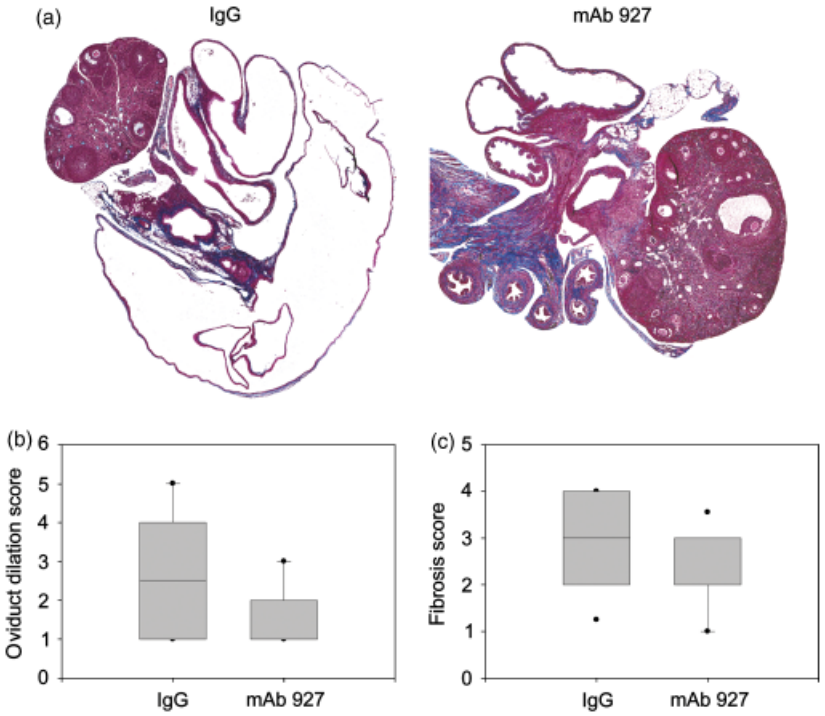

Fig. 4. pDC contribute to the sequelae of genital *Chlamydia muridarum* infection. GT were harvested 7 weeks after infection following treatment with the control antibody (IgG) or anti-BST2 (mAb 927) as described in Materials and methods. Trichrome slides were prepared as described in Materials and methods, and scanned with Aperio ScanScope. (a) Representative trichrome staining of oviducts at $\times 2$ using the SCANSCOPE software. (b) Oviduct dilation scoring measured by oviduct area using the SCANSCOPE software, with $n = 12$ for each group. (c) Fibrosis score as measured by collagen deposition scoring in the oviduct, with $n = 12$ for each group.

the clearance of infection, we found that mice depleted of pDC exhibited a significant reduction in oviduct dilation (Fig. 4a and b) as well as collagen deposition in the oviduct (Fig. 4b and c) compared with that seen in control mice. These data indicate that depletion of pDC results in less severe sequelae following genital *C. muridarum* infection.

Discussion

Recent evidence in the literature suggests that pDC are unique among DC subsets, particularly in their inability to induce effector responses against a microbial insult. This activity of pDC seemingly stems from the unique expression of surface molecules for antigen acquisition, a limited repertoire of receptors for microbial recognition (i.e. toll-like receptors), and the capacity to present antigen on the cell surface that is markedly less efficient than other DC subsets (Villadangos & Young, 2008). Our recent work suggested that pDC were not capable of inducing the protective Th1 response to genital chlamydial infection. The work here is in agreement with that supposition as well as recent findings in the literature demonstrating that pDC do not affect induction of effector responses to genital viral infection (Lund *et al.*, 2006). That pDC depletion altered neither the induction of a protective Th1 response nor the clearance of infection indicates that pDC do not influence the induction of protective T-cell responses to genital infection by *C. muridarum*.

Tolerogenic responses during genital chlamydial infection are not well defined. Given our recent demonstration of the induction of pDC during genital chlamydial infection (Moniz *et al.*, 2009), and recent evidence demonstrating pDC as central factors in the maintenance of tolerance (Ochando *et al.*, 2006; Goubier *et al.*, 2008), we sought to determine whether pDC modulate tolerogenic responses during genital chlamydial infection. Treg appear to be central in effector-mediated maintenance of tolerance (Tang & Bluestone, 2008), yet the role of Treg during genital chlamydial infection has not been examined. In this work, we demonstrate a marked Treg response to genital chlamydial infection (Fig. 1), and further examined whether pDC played a role in the maintenance of this response by depletion of pDC during the course of genital chlamydial infection. Depletion of pDC resulted in a decrease in the number of Treg in the spleen, but not the site (GT) or the draining lymph node (ILN) of infection. These observations indicate that modulation of Treg numbers by pDC is limited to the spleen during chlamydial infection. However, our work utilized antibody-mediated depletion of pDC, which may not completely ablate pDC. It is possible that in the total absence of pDC, Treg numbers may be affected in the draining lymph nodes and site of chlamydial infection. Thus, further efforts are warranted to determine the role that pDC play in the modulation of Treg responses during

chlamydial infection using more absolute means of pDC ablation, such as that recently shown through constitutive and conditional deletion of the transcription factor E2-2 (Cisse *et al.*, 2008).

The role of T-cell subsets such as IFNγ-producing NKT and CD8 cells during genital chlamydial infection is not well described. In models of chlamydial infection of the lung, NKT have been clearly shown to be detrimental to the host by prolonging the bacterial burden and exacerbating the sequelae of infection (Bilenki *et al.*, 2005). This activity was shown, in part, to be due to the promotion of Th2 responses by NKT cells. Such a divergence from the protective Th1 response is thought to result in the sequelae of genital chlamydial infection (Brunham & Rey-Ladino, 2005), and as pDC have been shown to interact with NKT cells in other models of infection (Diana *et al.*, 2009), we examined the effect of pDC on NKT cells during genital chlamydial infection. Interestingly, we found that NKT cells were not detectable in either the ILN or the GT later in a primary infection (data not shown) and that their presence in the spleen during infection coincided with the production of IFNγ and not interleukin-4 (Fig. 3b, data not shown). Furthermore, we show that pDC depletion results in an increase in the numbers of IFNγ-producing NKT cells in the spleen. This suggests that pDC modulate the production of IFNγ by NKT cells, whose consequence is unclear and warrants both the further examination of NKT cell activity during genital chlamydial infection as well as the consequence of this activity.

In a manner similar to NKT cells, we found that pDC depletion resulted in higher numbers of IFNγ-producing CD8 T cells in the spleen. It is clear that CD8 T cells are not central in the clearance of genital chlamydial infection, but instead appear to be involved in the promotion of sequelae following chlamydial infection (Igietseme *et al.*, 2009). In mice, the sequelae of genital chlamydial infection are most apparent in the formation of hydrosalpinx and fibrosis in the oviducts. The cause of these pathologies is not well understood, but is associated with excessive inflammation (Cohen & Brunham, 1999; Brunham & Rekart, 2008), which is thought to promote the fibrosis implicated in the sequelae of infection. As the tolerogenic potential of pDC may include the regulation of inflammation, we anticipated pDC depletion to result in increased pathology. Surprisingly, depletion of pDC resulted in a decrease in the sequelae following genital chlamydial infection (Fig. 4), indicating that pDC contribute to the formation of pathology following chlamydial infection. Furthermore, the decrease in sequelae following pDC depletion is inconsistent with our finding that pDC increase the activity of CD8 T cells that are implicated in promoting pathology. The possible reason for this discrepancy is that our method of pDC depletion was incomplete in the GT (Fig. 2a), and that this incomplete depletion resulted in the lack of an effect on CD8 T cells in the GT following pDC depletion (data not shown). Again, these findings warrant further analysis of the role that pDC play during genital chlamydial infection in a model of complete pDC ablation (Cisse *et al.*, 2008).

Our finding that pDC depletion results in less severe pathology was unexpected, and raises the question as to how pDC promote the formation of sequelae. A recent study showed that pDC were associated with mucopurulent cervicitis and inflammatory factors in women with a chlamydial genital infection (Agrawal *et al.*, 2008). The mechanism of this contribution is unclear, but might include pDC-mediated modulation of either Treg or nonprotective T-cell subsets (CD8+ or NKT) as demonstrated in this work. Another possibility is that depletion of pDC altered the specific antigens to which T-cell responses are induced. As we observed no change in the course of infection, it would seem that such a scenario does not include responses to chlamydial antigens central to the clearance of infection (protective responses). Instead, pDC may modulate nonprotective, but pathologically relevant responses as we show with CD8 and NKT cells. Before such a possibility can be addressed, however, a more clear definition of the roles that both Treg and nonprotective T cells play during chlamydial infection is required. Following these analyses, a more thorough examination of the role that pDC play in the modulation of Treg and nonprotective T-cell responses may then lead to a clearer understanding of the role that pDC play during genital chlamydial infection.

In summary, we demonstrate that pDC are not central in the induction of protective Th1 responses during genital *C. muridarum* infection, but rather affect the numbers of Treg and nonprotective T cells in the spleen. We also provide evidence that pDC contribute to the sequelae of genital chlamydial infection, which may be the result of pDC modulation of Treg and nonprotective T cells. Together, these findings indicate that pDC play an unexpected role in the formation of sequelae following genital chlamydial infection that is independent of the clearance of infection.

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