The phenotype and function of naturally existing regulatory dendritic cells in nematode-infected mice

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Immunosuppression associated with chronic helminth infections has been documented in many studies and regulatory T (Treg) cells have been shown to mediate the nematode-induced immunosuppression, but the role of dendritic cells (DCs) in the induction of Treg cell response and immunosuppression has not yet been fully determined. We analysed the response and function of DCs in mesenteric lymph node (MLNs) of mice infected with a gastrointestinal nematode, *Heligmosomoides polygyrus*, and observed a substantial expansion of DCs in MLNs following the infection. The CD11c+ DCs in MLNs of infected mice showed reduced expression of co-stimulatory molecules CD40, CD86 and MHC-II, and production of inflammatory cytokines IL-12 and IL-6. Analysis of MLN DC subsets defined by CD11c and CD45RB expression showed that the CD11c+CD45RBmid subset increased rapidly following *H. polygyrus* infection and the CD11c+CD45RBhigh subset expanded from the third week after infection. In the co-culture of sorted DC subsets with ovalbumin-(OVA-)specific T cell receptor (TCR) transgenic CD4+ T cells, CD11clowCD45RBmid DCs induced a low proliferation response and a high level of IL-10 production in CD4+ T cells, whereas CD11cmidCD45RBhigh DCs induced more IFN-γ and IL-4 producing CD4+ T cells. Intracellular staining revealed that CD11clowCD45RBmid DCs promoted CD4+ Foxp3+ differentiations. These results indicate that nematode infections selectively induce expansion of the CD11clowCD45RBmid regulatory DC subset that promotes development of Foxp3+ and IL-10 producing Treg cells. The Treg cell responses and immunoregulatory cytokines induced by this regulatory DC subset in turn play an important role in mediation of the nematode-induced immunosuppression.

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1. Introduction

Parasitic nematode infections are prevalent in many regions of the world and represent one of the most important health problems. Infections with these parasites often induce Th2-dominated immune responses which have been shown to be important for control of the infections (Urban et al., 1992). Many studies in humans and in animal models, however, also demonstrate that infections with parasitic nematodes markedly modulate the host’s immune function, resulting in a suppressed immune response (Maizels et al., 2004). The nematode-induced immunosuppression is manifested as (i) a chronic course of nematode infections allowing their long-term survival (Taylor et al., 2005), (ii) impaired immunity to concurrent infection with unrelated pathogens or a reduced immune response to unrelated antigens (Su et al., 2005; Rausch et al., 2010), and (iii) protection of the host from autoimmune and allergic diseases (Schnoeller et al., 2008).

Many studies in recent years demonstrated that host nematode infections are often associated with increased responses of regulatory T (Treg) cells including Foxp3+ Treg cells, IL-10-producing Tr1 and TGF-β-producing Th3 CD4+ T cells (Finney et al., 2007; Rausch et al., 2009, 2010). These immune regulatory cells and cytokines are the major factors in mediating the nematode-induced immunosuppression (Doetze et al., 2000; Wilson et al., 2005; Van Riet et al., 2007; Grainger et al., 2010). However, the immunological mechanisms by which the nematode parasites induce Treg responses are not fully understood.

Dendritic cells (DCs), as a professional antigen presenting cell (APC), play important roles in initiating immune responses to infection and directing the polarisation of CD4+ Th helper cell responses. Depending on the type of antigen or pathogenic, the antigen dose and the priming conditions, DCs can induce the development of either CD4+ Th1 or Th2 responses (Sher et al., 2003; Kaiko et al., 2008). DCs may also play a role in induction of immune tolerance and certain subsets of DCs, such as immature or CD8α+ DCs, have been reported to preferentially promote Treg cell responses (Lutz...
and Schuler, 2002; Steinman et al., 2003; Yamazaki and Steinman, 2009; Zou et al., 2010). Coombes et al. (2007) reported a subset of MLN DCs expressing CD103 which preferentially induces the development of Foxp3+ Treg cells. Several studies also demonstrated the existence of a distinct subset of regulatory DCs characterised by expression of low levels of CD11c and high levels of CD45RB. These CD11c<sub>low</sub>CD45RB<sub>high</sub> DCs are present in spleens and lymph nodes in normal mice and increased in frequency in transgenic mice expressing high levels of IL-10 (Wakkach et al., 2003). Furthermore, stimulation of bone marrow cells cultured with immunosuppressive neuropeptides, the vasoactive intestinal peptide and the pituitary adenylate cyclase-activating polypeptide, promotes the differentiation of bone marrow cells into CD11c<sub>low</sub>CD45RB<sub>high</sub> DCs (Delgado et al., 2005). The CD11c<sub>low</sub>CD45RB<sub>high</sub> DCs generated in both IL-10 transgenic mice and induced by neuropeptides secrete high levels of IL-10 in vitro and are able to induce a functional Treg response in vitro and in vivo (Wakkach et al., 2003; Delgado et al., 2005).

Nematode parasite infections, or treatment with products from the parasites, are able to modify the phenotypes and functions of DCs (Chen et al., 2006; Hamilton et al., 2009; Langelaar et al., 2009), but the underlying mechanisms were not clearly defined. In the present study, we investigated the phenotypes and functions of DC populations in mice during infection with a murine intestinal nematode parasite, Heligmosomoides polygyrus, and observed the existence of different DC subsets defined by expression profiles of CD11c and CD45RB. Functional analysis revealed a regulatory DC subset that expresses low levels of CD11c, intermediate levels of CD45RB and promotes a Treg response.

2. Materials and methods

2.1. Mice and parasites

Female BALB/c mice, 6- to 8-week-old, were purchased from Vital River Laboratories (Beijing, China). DO11.10 ovalbumin-(OVA-)specific T cell receptor (TCR) transgenic male mice were obtained from Nanjing University Model Animal Research Center (Nanjing, China). Heligmosomoides polygyrus parasites were kindly provided by Dr. M. Scott (McGill University, Montreal, Canada) and maintained in BALB/c mice as previously described (Su et al., 2005). Heligmosomoides polygyrus infection in mice was initiated by oral inoculation of 300 L3s. For OVA immunisation, naive mice and the mice infected with H. polygyrus for 2 weeks were injected s.c. with 20 µg OVA (Calbiochem, Darmstadt, Germany) emulsified in FCA. Four weeks after immunisation, mice were bled and serum samples were prepared. Mice were housed in the animal facility of the Guangzou Institutes of Biomedicine and Health under specific pathogen-free conditions. All experiments with animals were carried out in accordance with the national animal protection guidelines and approved by the Institutional Animal Care and Use Committee.

2.2. Cell cultures

Spleens and MLNs from naive and infected mice were removed aseptically and the lymphoid organs were treated by collagenase digestion as described by others (Inaba et al., 1998). Single-cell suspensions were prepared in RPMI 1640 medium (Invitrogen, Shanghai, China) supplemented with 10% heat-inactivated FCS (HyClone, Beijing, China), 25 mM HEPES, 0.12% gentamicin and 2 mM glutamine (complete medium, CM). Red blood cells were lysed with 0.175 M NH₄Cl. Total cell numbers were recorded for individual lymphoid organs and the viability of the cells (always >95%) was determined by trypan blue exclusion. Aliquots of 1 ml cell suspension at a concentration of 5 × 10⁵ cells/ml were cultured in 48-well culture plates in CM in the presence or absence of H. polygyrus adult worm antigen (20 µg/ml) prepared as previously described (Su et al., 2005). The cultures were incubated for 48 h at 37 °C in a humidified CO₂ incubator. Supernatants were collected and stored at −20 °C for the cytokine assay.

2.3. DC and CD4<sup>+</sup> T cell isolation and co-culture

Spleen and MLN single cell suspensions were prepared as described in Section 2.2 and CD11c<sup>+</sup> DCs were enriched by positive selection with anti-CD11c magnetic beads (Miltenyi Biotech, Auburn, CA, USA) according to the manufacturer's instructions. The enriched DCs were typically of >90% purity as determined by flow cytometry. For isolation of DC subsets, the DCs enriched by magnetic beads were incubated with anti-CD11c/CD3 monoclonal antibodies (mAbs) to block the Fc receptors, and doubly stained with anti-CD11c-FITC and anti-45RB-APC (both from ebioscience, San Diego, CA, USA). The cells were sorted by FACSAria (BD Biosciences, Shanghai, China). To isolate CD4<sup>+</sup> T cells, spleen cells were first labelled with anti-CD11c magnetic beads and passed through columns to remove the CD11c<sup>+</sup> DCs. Cells in the cell-thorowere collected, washed, labelled with anti-CD4 magnetic beads and enriched by positive selection (Miltenyi Biotech). The enriched CD4<sup>+</sup> T cells had >95% purity.

DCs were cultured at a concentration of 1 × 10⁶/ml in CM in the presence of 1 µg/ml LPS (lipopolysaccharide) (Sigma–Aldrich, Louis, MD, USA) or 1 mM CpG (oligonucleotide containing-Cytosine-phosphate-Guanine-motifs) (Invivogen, CA, USA) for 24 h and supernatants were harvested for cytokine analysis. For proliferation assays, 4 × 10⁴/well CD4<sup>+</sup> T cells were co-cultured in a 96-well U-bottom plate with three different numbers of DCs (DC:T ratios 1:1, 1:3 and 1:9) in the presence of 1 mg/ml ovalbumin (OVA) (Calbiochem, Shanghai, China) for 72 h. One µCi/well of ³H-thymidine was added during the last 18 h. Incorporation of ³H-thymidine by the cells was determined by scintillation counting. For determination of cytokine production in DC and CD4<sup>+</sup> T cell co-culture, 4 × 10⁴ DCs and 2 × 10⁵ CD4<sup>+</sup> T cells were co-cultured in U-bottom plates in the presence of 1 mg/ml OVA for 72 h. Supernatants were harvested for cytokine analysis by ELISA. For intracellular cytokine staining, 10 ng/ml phorbol 12-myristate 13-acetate (PMA) (Calbiochem, Shanghai, China) and 500 ng/ml ionomycin (both from Enzo LifeSciences, PA, USA) were added to the co-cultures during the last 5 h of culture and brefeldin A (BFA) was added 4 h before harvesting the cells. To analyse the Foxp3<sup>+</sup> Treg response induced by subsets of DCs, 4 × 10⁵ purified DCs and 2 × 10⁵ CD4<sup>+</sup> T cells purified from spleens of DO11.10 mice were co-cultured in U-bottom plates in the presence of 1 mg/ml OVA for 72 h. The cells were harvested and stained with a Treg cell staining kit (as described in Section 2.4) for flow cytometric analysis.

2.4. Flow cytometry

For analysis of DCs, MLN single cell suspensions were first incubated with anti-CD16/CD32 monoclonal antibodies (mAbs). After washing, the cells were stained with anti-CD11c-FITC, anti-CD45RB-APC, anti-CD40-Phycoerythrin (anti-CD40-PE), anti-CD80-PE, anti-CD86-PE and anti-MHC-II-PE fluorescent mAbs (all from ebioscience) following standard protocols. Isotype-matched mAbs were used as staining controls. To determine the frequencies of CD4<sup>+</sup> Foxp3<sup>+</sup> Treg cells, cell samples were stained with a mouse Treg cell staining kit (ebioscience) following the manufacturer's instructions For intracellular IFN-γ, IL-4 and IL-10 staining, cells were collected from the DC-CD4 T cell co-cultures and stained with intracellular cytokine staining kits (BD Biosciences) as instructed by the manufacturer. Flow cytometric analysis was performed on
using CellQuest software (version 6.3). a FACSAria (BD Biosciences, Beijing, China) and data were analysed in a FACSAria (BD Biosciences, Beijing, China) and data were analysed using CellQuest software (version 6.3).

2.5. Cytokine and antibody ELISA

Levels of IL-4, IL-6, IL-10, IL-12p40, IFN-γ and TNF-α in cell culture supernatants were determined using an ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Serum levels of OVA-specific antibodies were determined by ELISA as previously described (Su et al., 2005). Briefly, ELISA plates were coated with OVA antigen overnight at 4 °C and subsequently blocked with 1% BSA in PBS for 1.5 h. After washing, serially 2-fold diluted serum samples were added and incubated for 1 h at room temperature. After extensive washing, horseradish peroxidase-conjugated goat anti-mouse total Ig, IgG1 and IgG2a antibodies (Southern Biotechnology Associates, Birmingham, AL, USA) were added and incubated at room temperature for 1 h. Reactivity was visualised by the addition of substrate and O.D. values were read in a microplate reader. Antibody levels were expressed as ELISA titres, the reciprocal of the highest dilution of serum that yields the background O.D.

2.6. Statistical analyses

Statistical analyses were performed with GraphPad Prism software (San Diego, USA). Significance of differences between groups was analysed using the Student's t test. To compare the cytokine and DC subset responses during the course of infection with the values of naïve mice, one-way ANOVA with Dunnett’s post-test were used to determine the significance of the difference. Data are presented as mean ± S.D. A P value less than 0.05 was considered significant.

3. Results

3.1. Immunosuppression and Treg responses in H. polygyrus infection

To determine the immunoregulatory effects of H. polygyrus infection, we first analysed the antibody response to OVA immunisation in the nematode-infected mice. Naïve mice and mice infected with H. polygyrus for 2 weeks were immunised with OVA antigen in CFA and the OVA-specific antibody response was analysed. Although the two groups of mice showed comparable levels of OVA-specific total Ig and IgG1 antibodies, the mice harbouring nematode parasites produced lower levels of OVA-specific IgG2a antibody compared with mice immunised with OVA alone (Fig. 1A).

We then analysed the responses of T cells and cytokines that might be involved in immune regulation of H. polygyrus infection. Flow cytometric analyses showed that, in MLNs of uninfected mice, approximately 8–9% of CD4+ T cells were positive for Foxp3, a marker for Treg cells. Following nematode infection, the frequency of Foxp3+ Treg in total CD4+ T cells increased rapidly and significantly, and the high level was maintained between 1 and 5 weeks p.i. The frequency of Foxp3+ Treg cells declined gradually from weeks 4 to 6 p.i. (Fig. 1B). No significant increase in Foxp3+ Treg cell frequency was observed in spleens of mice following H. polygyrus infection. To determine the cytokine response to H. polygyrus infection, spleen and MLN cells were cultured and the levels of cytokines in supernatants were determined. Both spleen and MLN cells from nematode-infected mice produced, as expected, strong responses of Th-2 associated cytokines IL-4 (Fig. 1C) and IL-13 (data not shown) in response to stimulation with H. polygyrus antigen (Hp-Ag) in vitro. The immune cells from nematode-infected mice also produced high levels of IL-10 (Fig. 1D). However, H. polygyrus infection did not induce an increased production of Th-1-associated cytokine IFN-γ (data not shown). These results indicate that H. polygyrus infection induces not only Th-2 cytokine production but also Foxp3+ Treg and immunoregulatory cytokine IL-10 responses.

3.2. Phenotypical changes of DCs in H. polygyrus infection

We then analysed the phenotypical and functional changes of CD11c+ DCs in the MLNs of naïve and H. polygyrus-infected mice. Infection with H. polygyrus induces enhanced cellularity in MLNs. At weeks 2 and 3 of infection, the numbers of MLN cells increased by 9- and 15-fold over the levels of uninfected mice, respectively.
The total number of CD11c+ DCs also increased in MLNs following infection but the frequency of CD11c+ DCs in the MLNs increased transiently 1 week after infection (Fig. 2A). Further analysis of MLN DCs by flow cytometry showed that total CD11c+ DCs from *H. polygyrus*-infected mice expressed lower levels of co-stimulatory molecules CD40, CD80 and MHC-II antigens compared with DCs from naive mice (Fig. 2B and C). CD11c+ DCs were enriched from MLNs of naive and infected mice by magnetic sorting, cultured in vitro; and cytokine production in response to antigen and analysed by flow cytometry. We observed that the R1 subset rose 3 weeks after infection and remained at high levels at 2 weeks p.i. and declined thereafter. The percentage of the R3 sub-population increased following nematode infection, peaked between naive and infected mice (Fig. 2D).

3.3. Characteristics of DC subsets in MLNs in naïve and H. polygyrus-infected mice

The results obtained from analysis of total CD11c+ DCs described above suggest that the phenotype and function of DCs of local draining lymph nodes were modified during the infection with *H. polygyrus*. It is not known whether these phenotypical and functional changes observed in total DCs represent alteration of the sub-populations of the DCs. To address this question, we doubly stained the MLN DCs from naïve and nematode-infected mice with fluorescent mAbs against CD11c and CD45RB, and analysed those by flow cytometry. Four distinct subsets were observed, which are defined by expression levels of CD11c and CD45RB: CD11clowCD45RBmid (R1), CD11chighCD45RBmid (R2), CD11clowCD45RBhigh (R3) and CD11chighCD45RBflow (R4) (Fig. 3A). Following *H. polygyrus* infection, the frequencies of the four DC subsets changed in different ways. The frequency of the R1 DC sub-population increased following nematode infection, peaked at 2 weeks p.i. and declined thereafter. The percentage of the R3 subset rose 3 weeks after infection and remained at high levels up to 5 weeks p.i. In contrast, in nematode-infected mice the frequencies of R2 and R4 declined (Fig. 3B).

To further characterise the four DC subsets defined by CD11c and CD45RB expression, MLNs were collected from mice 3 weeks after nematode infection and DCs were triple stained with fluorescent antibodies to CD11c, DC45RB and CD40, CD80, CD86 or MHC-II antigen and analysed by flow cytometry. We observed that the R1
Fig. 3. Distinct subsets of dendritic cells (DCs) from mesenteric lymph nodes (MLNs) in uninfected and *Heligmosomoides polygyrus* (Hp)-infected mice, defined by expression levels of CD11c and CD45RB molecules. (A) Distribution and changes in percentages of R1, R2, R3 and R4 subsets of DCs from MLNs of uninfected and nematode-infected (3 weeks) mice. (B) Changes in proportion of the four subsets of MLN DCs during the course of Hp infection (*P* < 0.05, **P** < 0.01 compared with naïve mice). Results are presented as mean ± S.D. of three mice per group. Data shown are from one of two experiments.

Fig. 4. Co-stimulatory molecule expression, cytokine production and T cell proliferation induced by R1, R2 and R3 dendritic cell (DC) subsets and total mesenteric lymph node (MLN) DCs in *Heligmosomoides polygyrus* (Hp)-infected mice. (A) Expression levels of CD40, CD80, CD86 and MHC-II by four subsets of MLN DCs from mice three weeks after nematode infection. (B) Levels of IL-12p40, TNF-α, IL-6 and IL-10 produced by three subsets of DCs and total MLN DCs stimulated in vitro by CpG oligonucleotide and lipopolysaccharide (LPS) (*P* < 0.05, **P** < 0.01 compared with the levels of total MLN DCs). (C) Proliferation responses of CD4+ T cells from DO11.10 mice co-cultured with three subsets of DCs and total MLN DCs (*P* < 0.05, **P** < 0.01 compared with the levels induced by total MLN DCs). Results are presented as mean ± S.D. of triplicate wells. Data from one of two to three experiments with similar results are shown.
and R3 DC subsets expressed lower levels of CD40 and CD86 molecules compared with the other two subsets. The R3 subset expressed a lower level of CD80 and R1 showed a reduced level of the MHC-II molecule. Among the four DC subsets, the R2 subset showed the highest levels of the co-stimulatory molecules and MHC-II antigen (Fig. 4A). We then purified R1, R2 and R3 DC subsets by fluorescence-activated cell sorting (FACS) (we were unable to collect the R4 subset due to its scarcity) and examined their cytokine production profiles. The purified DC subsets and unfractioned MLN DCs were cultured in the absence or presence of LPS and CpG, and the secretion of cytokines in supernatants was analysed. The R1 MLN DCs were cultured in the absence or presence of LPS and CpG, and the secretion of cytokines in supernatants was analysed. The R1 DCs produced minimal levels of IL-12p40, TNF-α, IL-6 and IL-10 in the medium control and in response to stimulation with LPS and CpG (Fig. 4B). DCs of the R2 subset stimulated by LPS produced significantly increased levels of the four cytokines compared with the total CD11c+ DC from MLNs, but intermediate levels of cytokines in response to stimulation with CpG but minimal levels of cytokines when stimulated with LPS (Fig. 4B). To determine the ability of the three DC subsets to activate CD4+ T cells, the purified DCs were co-cultured with CD4+ T cells from DO11.10 mice in the presence of OVA antigen. We observed that R2 DCs induced strong CD4+ T cell proliferation but R1 DCs failed to induce CD4+ T cell activation and proliferation. R3 DCs induced intermediate levels of T cell proliferation (Fig. 4C). These results demonstrate that the R1, R2 and R3 are distinct DC subsets in terms of cytokine secretion, response to stimuli and their ability to activate CD4+ T cells.

3.4. CD4+ T cell response and polarisation driven by three DC subsets

The apparent difference in phenotypes and function between the three subsets of DCs suggests that these DC subsets may play differential roles in driving the CD4+ T cell polarisation and immune responses. To test this, the purified DC subsets were co-cultured in vitro with CD4+ T cells of DO11.10 mice and T cell responses were analysed. CD4+ T cells co-cultured with R1 DCs produced lower levels of IFN-γ, IL-13, IL-6 and IL-10 but a higher level of IL-10 compared with T cells co-cultured with unfractioned total MLN DCs. CD4+ T cells incubated with R2 DCs produced more IL-6 but less IL-13 and IL-10. R3 DCs promoted IL-13 and IL-10 production but suppressed IL-6 secretion by CD4+ T cells (Fig. 5A). No significant level of IL-4 in cell culture supernatant was detected by ELISA. To further characterise the polarisation of CD4+ T cells by different DC subsets, CD4+ T cells were stained for intracellular IFN-γ, IL-10 and IL-4, and analysed by flow cytometry (Fig. 5B). It was observed that, in comparison with the CD4+ T cells co-cultured with total MLN DCs, T cells co-cultured with R3 DCs showed significantly higher levels of IFN-γ and IL-4. In CD4+ T cells co-cultured with R1 DCs, a significantly increased percentage of IL-10 positive cells was detected, albeit slight increases in frequencies of IL-4 and IFN-γ positive cells were observed. CD4+ T cells incubated with R2 DCs did not show significant changes in IL-4 and IL-10 positive cells, and the frequency of IFN-γ producing cells was reduced in R2 DCs. However, when the CD4+ T cells were co-cultured with R1 DCs, significantly increased frequency of Foxp3 positive cells was detected in the CD4+ T cells. DCs in R2 and R3 subsets did not induce increased expression of Foxp3 in CD4+ T cells (Fig. 6). These results reveal the functional difference of the three DC subsets examined and, more importantly, the R1 DC is tolerogenic DC subset as demonstrated by its ability to promote IL-10 production by CD4+ T cells and Foxp3+ T cell differentiation.
Table 1

Percentages of IFN-γ, IL-4 and IL-10 producing CD4+ T cells co-cultured with three dendritic cell (DC) subsets (R1–R3) and total unfractioned DCs from nematode-infected mice.

<table>
<thead>
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<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>Total MLN DCs</th>
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<tr>
<td>IFN-γ+</td>
<td>26.2 ± 0.2a</td>
<td>14.8 ± 0.2b</td>
<td>52.0 ± 0.4c</td>
<td>18.6 ± 0.2</td>
</tr>
<tr>
<td>IL-4+</td>
<td>11.0 ± 0.4a</td>
<td>3.1 ± 0.4</td>
<td>39.0 ± 0.0b</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>IL-10+</td>
<td>7.8 ± 0.9a</td>
<td>2.9 ± 0.5</td>
<td>4.9 ± 0.3</td>
<td>3.5 ± 0.3</td>
</tr>
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</table>

Data are presented as mean (±S.D.) of triplicate wells. Experiments were performed four times with similar results. A statistically significant difference in comparison with total mesenteric lymph node (MLN) DCs:

- \( P < 0.05 \)
- \( P < 0.01 \)
- \( P < 0.001 \)

4. Discussion

It has been well established that parasitic nematode infections markedly modulate the host’s immune system, often resulting in immunosuppression (Hatmann and Lucius, 2003; Maizels et al., 2004). This is believed to be a means for parasites to evade the host’s immune attack to ensure their survival. Several studies have demonstrated that infection with *H. polygyrus* induces the response of Foxp3+ Treg cells and production of immunosuppressive cytokines (Wilson et al., 2005; Finney et al., 2007; Rausch et al., 2009, 2010; Grainger et al., 2010), but the role of DCs in the induction of Treg cell and immunoregulatory cytokine is not clearly defined. In the present study, we analysed the DC populations in MLNs of mice infected with *H. polygyrus* and observed a subset of R1 R2 R3 Total MLN-DCs + Foxp3 CD4+ (%)

<table>
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<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>Total MLN-DC</th>
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<tr>
<td></td>
<td>17.2</td>
<td>4.9</td>
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**Fig. 6.** Induction of Foxp3 protein expression in DO11.10 mouse CD4+ T cells co-cultured with the dendritic cell (DC) subsets and total mesenteric lymph node (MLN) DCs. (A) Representative flow cytometric plots. (B) Mean percentage of Foxp3+ Treg cells in total CD4+ T cells co-cultured with three DC subsets and total MLN DCs. (**P < 0.01 compared with the levels induced by total MLN DCs). Results are presented as mean ± S.D. of triplicate wells. Experiments were performed three times with similar results.
and mice with a thermal injury (Wong and Rodriguez, 2008; Liu et al., 2010). To investigate the role of this regulatory DC subset in nematode-induced immunosuppression, we analysed the profiles of CD11c and CD45RB expression on DCs in MLNs from mice infected with H. polygyrus. We observed four distinct DC subsets in MLNs that are defined by the expression profiles of CD11c and CD45RB (Fig. 3). Further analysis revealed that the CD11clowCD45RBhigh DC subset expanded rapidly following H. polygyrus infection and expressed lower levels of CD40, CD86 and MHC-II antigen, and produced very low levels of IL-12p40, TNF-α, IL-6 and IL-10 in vitro in response to stimulation by CpG and LPS compared with other subsets of DCs. Importantly, this subset of DCs was able to promote CD4+ T cell IL-10 production and FoXP3+ Treg cell differentiation. These results demonstrate that H. polygyrus infection induces the expansion of a naturally existing regulatory DC subset in MLNs that promotes the Treg response. In this nematode infection model, the regulatory DC functions are identified in a CD11clowCD45RBhigh DC subset that is different from the CD11clowCD45RBmid regulatory DCs observed in spleens as reported in previous studies (Wakkach et al., 2003; Wong and Rodriguez, 2008; Liu et al., 2010). This is probably due to the difference in lymphoid tissues (MLN in our study versus spleen in others) and/or the experimental models studied.

Three of the four DC subsets (R1, R2 and R3) also displayed different immunological features. The CD11clowCD45RBmid DC subset (R1) in MLNs expanded rapidly following nematode infection and its frequency peaked 2 weeks p.i. This DC subset also expressed reduced levels of CD40, CD86 and MHC-II antigen compared with other three DC subsets, and induced poor CD4+ T cell proliferation in vitro. Importantly, this DC subset promoted IL-10 production and FoXP3+ Treg differentiation. The increased frequency of FoXP3+ Treg cells detected in the CD4+ T cell co-cultured with the CD11clowCD45RBmid DC subset is probably due to the conversion of FoXP3- T cells into FoXP3+ Tregs, but not the expansion of natural endogenous FoXP3+ Tregs, because no significant proliferation was observed in the CD4+ T cells when those were co-cultured with CD11clowCD45RBmid DCs (Fig. 4C). The percentage of the CD11clowCD45RBmid DC subset (R2) declined initially and returned to the level of naïve mice 5 weeks p.i. This DC subset induced the strongest T cell proliferation but showed poor ability to induce IFN-α, IL-4 and IL-10 cytokine production by CD4+ T cells. The CD11clowCD45RBmid DCs (R3) expanded significantly only 3 weeks p.i. and induced intermediate levels of CD4+ T cell proliferation. Interestingly, this subset of DCs activated CD4+ T cells to produce high levels of IFN-γ and IL-4, suggesting that they consist of DC1s and DC2s with the ability to induce Th-1 and Th-2 effector T cell responses, respectively.

Although it is often stated in the literature that the Th-2 cytokines induced during helminth infections might be the major factors responsible for immunosuppression, no conclusive evidence exists to support this notion. Instead, many studies demonstrate that the Treg cells, including FoXP3+ cells and IL-10 and TGF-β cytokines, are the major factors mediating immunosuppression in helminth infections (Doetze et al., 2000). It was also reported that H. polygyrus infection prevented allergic airway inflammation in a murine asthma model and the preventative effect is mediated by Treg cells (Wilson et al., 2005). We previously showed that concurrent infection of mice with H. polygyrus and the murine malaria parasite Plasmodium chabaudi impaired immune protection against malaria (Su et al., 2005). Neutralisation of IL-4 and IL-13 with mAbs in vivo in nematode and malaria co-infected mice failed to abrogate nematode-induced immunosuppression, but blocking IL-10 and depletion of CD25+ T cells prevented impairment of the immune response to malaria (our unpublished observation). These studies indicate that Treg cells and the immunosuppressive cytokine IL-10 play major roles in mediating nematode-induced immunosuppression. The CD11clowCD45RBmid regulatory DCs described in the present study link nematode infection with the development of Treg cell response and consequent immunosuppression.

In conclusion, we have described a DC subset in MLNs characterised by expression of a low level of CD11c and intermediate level of CD45RB that expand in response to infection with the nematode parasite H. polygyrus and display a regulatory function to induce a Treg cell response. This finding provides insight into the mechanism by which nematode parasites induce immunosuppression.

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