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This information is current as of September 23, 2015.

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J Immunol published online 11 September 2015
<http://www.jimmunol.org/content/early/2015/09/11/jimmunol.1403240>

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IL-4–Producing Dendritic Cells Induced during *Schistosoma japonica* Infection Promote Th2 Cells via IL-4–Dependent Pathway

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Although dendritic cells (DCs) have been widely demonstrated to play essential roles in initiation of Th2 responses in helminth infections and allergic reactions, the mechanisms remain uncertain largely because DCs do not produce IL-4. In present investigation, we have uncovered a novel subset of DCs from mice infected with Th2-provoking pathogens *Schistosoma japonica*, which independently promoted Th2 cells via IL-4–dependent pathway. These DCs contained similar levels of IL-4 mRNA and higher levels of IL-12p40 mRNA comparing to basophils, correlating to their Th2-promoting and Th1-promoting dual polarization capacities. Characterized by expression of FcεRI⁺, these DCs were induced independent of T cells. Further investigations revealed that Th2-promoting FcεRI⁺ DCs were monocyte-derived inflammatory DCs, which were sufficient to induce Th2 cells in vivo. Egg Ags together with GM-CSF or IL-3 alone were able to stimulate the generation of Th2-promoting FcεRI⁺ DCs from bone marrow cells in vitro. To our knowledge, our data for the first time demonstrate that IL-4–producing DCs are induced under some Th2-provoking situations, and they should play important roles in initiation of Th2 response. *The Journal of Immunology*, 2015, 195: 000–000.

Dendritic cells (DCs) are the most important APCs in orchestrating appropriate immune response via their superior abilities to stimulate naive T cell proliferation and to govern proper helper CD4⁺ T cell (Th) differentiation in response to invading pathogens. The polarizing cytokines generated by DCs play critical roles in determining Th differentiation when DCs engage with and activate naive CD4⁺ T cells. For example, DCs infected with intracellular pathogens like *Mycobacterium tuberculosis* produce polarizing cytokine IL-12 to promote a strong IFN-γ–producing Th1 response, which results in enhanced macrophage activation to resolve intracellular bacterial invasion (1). Infection with helminths results in Th2 responses which lead to production of Th2 cytokines including IL-4, IL-5, IL-9, and IL-13. Th2 responses contribute to control helminth infection via effector responses including IgE- and eosinophil-mediated destruction of pathogens. In addition to helminths, allergens can also induce Th2 response. Conditional ablation of

DCs in CD11c-DTR mice demonstrates that DCs are required to promote Th2 responses in allergen-induced asthma (2), in alum-boosted adjuvant activities (3), and in worm infection by *Schistosoma masoni* (4). Meanwhile, studies have also indicated that distinct specialized DC subsets control the development of Th2 cells in response to different Th2-associated Ags (5). However, neither conventional CD11c-positive DCs nor these identified specialized DC subsets were found to produce IL-4, the most potent polarizing cytokine for Th2 differentiation as well as the hallmark cytokine for Th2 cells (6). Therefore, the nature of the polarization factors from DCs to induce Th2 responses remains unclear.

In this study, we have investigated this long-sought-after answer on whether DCs can produce IL-4 in mice infected with helminth *Schistosoma japonica*, a trematode strain that is epidemic in Asia. Similar to *S. masoni*, this intravascular trematode remarkably provokes Th2 response and Th2-associated type 2 inflammatory pathologies during egg-stage infection both in mice and in humans (7). Furthermore, studies performed on animals infected by *S. masoni* or *S. japonica* have revealed critical roles played by IL-4 or IL-4R–activated molecules in the induction of Th2 response and its resultant type 2 inflammatory pathologies (8–10). Although basophils have been reported to act as APCs important for initiation of Th2 responses via IL-4 (11–13), studies on induction of Th2 response in *S. masoni* infection in mice have clearly demonstrated that basophil is dispensable for egg Ag-induced Th2 differentiation. Instead, an irreplaceable role of DCs is supported in this model (4). However, the driving force for Th2 induction by DCs is not clarified in schistosome infection.

Taking advantage of profusely infiltrated non-B non-T cells (NBNT) in the spleen in mice infected by *S. japonica*, we uncovered a novel subset of DCs that are induced during egg-stage infection, which can produce IL-4 and subsequently polarize Th2 differentiation via IL-4–dependent fashion. The phenotype of this novel DC subset is CD11c⁺CD49b⁺FcεRI⁺. Contrary to conventional CD11c-positive splenic DCs, this DC subset is monocyte-derived inflammatory DCs, which can be driven by IL-3 but not by

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Received for publication December 30, 2014. Accepted for publication August 3, 2015.

This work was supported by National Natural Science Foundation of China Grants 81071382 and 81273326.

Y.-L.M., L.Y., and X.-P.C. designed research; Y.-L.M., F.-J.H., L.C., W.-C.G., H.-M.B., J.L., N.-Y.Z., and X.-L.J. performed research; Y.-L.M., C.-X.C., and L.Y. analyzed data; and Y.-L.M., L.Y., and X.-P.C. wrote the paper.

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The online version of this article contains supplemental material.

Abbreviations used in this article: BM, bone marrow; DC, dendritic cell; DT, diphtheria toxin; HDM, house dust mite; NBNT, non-B non-T cell; SjEA, soluble *Schistosoma japonica* egg Ag; SmEA, egg Ag from *Schistosoma mansoni*; SWA, *Schistosoma japonica* worm Ag; WT, wild-type.

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GM-CSF from bone marrow (BM) cells *in vitro*, although IL-3 appears not to be required for its induction *in vivo*. Neither T cell is required for its induction. More remarkably, soluble egg Ags from *S. japonica* (SjEA) are unable to condition conventional DCs to induce Th2 response but rather stimulate the development of BM cells into Th2-promoting FcεRI-positive DCs. To our knowledge, our work for the first time demonstrated that IL-4-competent DCs can be induced by strong Th2-provoking pathogens like *S. japonica*, which provides a paradigm that DCs polarize Th2 responses via generation of polarizing cytokine IL-4.

Materials and Methods

Mice and infection

Wild-type (WT) BALB/c mice and C57/B6 mice were purchased from SLAC Laboratory (Shanghai, China). Line of IL-4-deficient mice (stock number 002496) and CD11c-DTR/EGFP mice (stock number 004512) were purchased from Jackson Mice; line of DO11.10 OVA-TCR transgenic mice (C.Cg-Tg(DO11.10)10Dlo/JNju) and RAG1-deficient mice (B6.129S7-Rag1^{tm1Mom}/JNju) were purchased from Model Animal Research Center of Nanjing University. GFP mice were provided by Dr. D. Changsheng from School of Life Science Tongji University. All mice were housed and bred under specific pathogen-free conditions in the Animal Center of Tongji University and used at 6–8 wk of age. Gender- and age-matched mice were infected percutaneously with 30 cercariae of *S. japonica*, which were shed from infected *Oncomelania hupensis* snails provided by National Institute of Parasitic Diseases at Shanghai, China. In some experiments, WT BALB/c mice were infected with 7×10^4 *Candida albicans* (SC5314) via tail vein injection. All procedures performed on animals within this study were approved by the Committee on the Ethics of Animal Experiments of Tongji University (permit number 11050096).

Flow cytometry analysis and cell isolation

Splenocytes from infected spleen were suspended and washed with cold FACS buffer and incubated with normal mouse serum on ice. To detect FcεRI-positive cells, splenocytes were stained with FITC-labeled anti-mouse CD3ε (145-2C11; BioLegend) and anti-mouse B220 (RA3-6B2; BioLegend), APC-labeled anti-mouse FcεRIα (MAR-1; BioLegend), PE-labeled anti-mouse CD11c (N418; BioLegend) and PE-Cy7-labeled anti-mouse CD49b (DX5; eBioscience), and relative populations were examined in non-B non-T cells. To sort basophils (CD11c⁺CD49b⁺FcεRI⁺), FcεRI-positive DCs (CD11c⁺CD49b⁺FcεRI⁺ and CD11c⁺CD49b⁺FcεRI⁺) and splenic conventional DCs (CD11c⁺FcεRI⁺), pooled splenocytes from three to five mice of 5 wk postinfection were firstly depleted of T and B cells using biotinylated anti-mouse CD3 (145-2C11; BioLegend) and anti-mouse B220 (RA3-6B2; BioLegend) plus avidin magnetic bead (BD Biosciences). Flow cytometry isolation was performed after cells being stained with FITC-labeled anti-mouse CD49b (DX5; eBioscience), PE-labeled anti-mouse CD11c, APC-labeled anti-mouse FcεRIα. The sorted cells were routinely back-checked using flow cytometry and their purities were ≥95%. Appropriately fluorescence-labeled mAbs were used to profile surface expression of CD11b (M170), Ly-6C (HK1.4), c-kit (2B8), siglec-F (E50-2440, BD), CCR7 (4B12), CD8 (53-6.7), MHC class II (M5/114.15.2), CD40 (1C10), CD80 (16-10A1), CD86 (GL-1), and IgE (RME-1). All the staining Abs were bought from Biolegend except otherwise noted.

BM cells were flushed from femurs. After lysis of RBCs, cells were cultured in the presence of 30 ng/ml IL-3 (PeproTech) for 12 d. In separate experiments, BM monocytes were sorted from BM cells as CD11c⁺FcεRI⁺CD11b⁺Ly-6C^{hi} (purity ≥ 95%) and cultured in the presence of 30 ng/ml IL-3 for 9 d. To test whether SjEA or *S. japonica* worm Ag (SWA) could induce the development of FcεRI-positive DC, BM cells were cultured in the presence of 25 ng/ml GM-CSF (PeproTech) and different concentration (0.1, 1, 5, 10, 20, and 50 μg/ml) of SjEA prepared as described (14), OVA or SWA for 5 d. Differentiated BM cells were stained with FITC-labeled anti-mouse CD49b, PE-labeled anti-mouse C-kit, Percp-Cy5.5-labeled anti-mouse CD11c, and APC-labeled anti-mouse FcεRIα to determine and sort relative cell populations of interest.

Naive CD4⁺ T cells were sorted from the spleen of DO11.10 mice using FITC-labeled anti-mouse TCR DO11.10 (KJ1-26; eBioscience), PE-labeled anti-mouse CD62L (MEL-14; BD Biosciences) and Percp-Cy5.5-labeled anti-mouse CD4 (GK1.5; BioLegend). The purity of the sorted naive CD4⁺ T cells was ≥99%.

Cell sorting was performed on FACS Aria II (BD Biosciences); flow cytometry analysis was performed on FACSVerse (BD Biosciences). Flow cytometry data analysis was analyzed by FlowJo software (FlowJo China).

Cell morphology

Highly purified basophils (CD11c⁺CD49b⁺FcεRI⁺), CD11c⁺CD49b⁺FcεRI⁺ cells, CD11c⁺CD49b⁺FcεRI⁺ cells, and conventional DCs (CD11c⁺FcεRI⁺) from the spleen of 5 wk infection mice were mounted on a slide by cytospin centrifugation and fixed with methanol and then stained with Wright-Giemsa. The cell morphology was examined under microscopy.

Neutralization of IL-3

Twenty-one days post-*S. japonica* infection, mice were injected i.p. with monoclonal anti-mouse IL-3 Ab (MP2-8F8; BioLegend) at 10 μg/each with 1-d interval until 35 d postinfection. Control mice were injected with PBS.

Coculture of naive CD4⁺ T cells and APCs

DO11.10-derived naive CD4⁺ T cells (1×10^6 /ml) were incubated with infected spleen- or BM-derived distinct DC subsets (5×10^5 /ml) or basophils (5×10^5 /ml) in RPMI 1640 complete medium in the presence of OVA_{323–339} (10 μg/ml) in a 0.2-ml volume in round-bottom 96-well plates. After 72 h culture, supernatants were replaced with fresh medium containing 10 ng/ml recombinant human IL-2 (PeproTech). An additional 2–3 d later, intracellular cytokine staining was performed as described below to measure the polarization of Th cells.

In experiments measuring proliferation of naive CD4⁺ T cells, FACS-sorted naive CD4⁺ T cells from DO11.10 were labeled with CFSE (Invitrogen) before being cocultured with relevantly isolated DC subsets or basophils at different ratio in the presence of OVA (40 μg/ml). Three days later, cells were harvested and OVA-specific T cell proliferation was determined after gated on CD4⁺ T cells.

Intracellular cytokine staining

Analysis of intracellular cytokine expression was performed as described previously (15). Briefly, after cell expansion in culture, cells were stimulated with PMA (20 ng/ml; Sigma-Aldrich) and ionomycin (1 μg/ml; Sigma-Aldrich) for 5 h with addition of brefeldin A (10 μg/ml; Sigma-Aldrich) during the last 2 h. Intracellular cytokine staining was then conducted always with parallel staining with fluorophore- and isotype-matched Abs against cytokine. Fluorophore-labeled anti-mouse cytokine Abs (PE-labeled anti-mouse IFN-γ and IL-10, APC-labeled anti-mouse IL-4, IL-13, and IL-5) were all purchased from BioLegend. Fixable viability staining from eBioscience was added to exclude signals from dead cells. Then, intracellular cytokine signals were analyzed after gated on CD4⁺ T cells.

Adoptive transfer

Monocytes were sorted as CD11c⁺FcεRI⁺CD11b⁺Ly-6C^{hi} cells from BM cells of GFP mice. After being washed twice in sterile normal saline, cells were tail vein-injected into 28 d *S. japonica* infected or into uninfected C57BL/6 mice at cell number of 3×10^6 per mice. Two days later, splenocytes were obtained and analyzed for cells of interest using flow cytometry.

CD11c-DTR transgenic mice were injected i.p. with 4 ng/g diphtheria toxin (DT; Sigma-Aldrich) and were adoptively transferred with naive CD4⁺ T cells ($4–5 \times 10^6$) from DO11.10 mice 1 d before different populations of APCs were injected. Transferred APCs included basophils (CD11c⁺CD49b⁺FcεRI⁺), CD11c⁺CD49b⁺FcεRI⁺ cells, CD11c⁺CD49b⁺FcεRI⁺ cells, and splenic conventional DCs (CD11c⁺FcεRI⁺) sorted from spleen of 5 wk schistosome infected mice. These cells were pulsed with OVA_{323–339} (20 μg/ml) for 3 h at 37°C before they (5×10^5 /mice) were injected into the CD11c-DTR mice depleted of endogenous DCs, respectively. Five days later, splenocytes were isolated and restimulated with OVA_{323–339} (10 μg/ml) for another 4 d, and the concentration of IL-4 in the supernatant was detected using ELISA kit (eBioscience).

Endocytosis

Distinct DC subsets and basophils were sorted from the spleen of 5-wk *S. japonica*-infected mice, washed once with 1×PBS, resuspended in RPMI 1640 medium containing 10% FBS, and plated onto 96-well flat-bottom plate at 1×10^6 cells/ml and 0.2 ml/well. Alexa Fluor 488 labeled OVA (Invitrogen) was added to the wells at a final concentration of 40 μg/ml, and the 96-well plates were incubated for 5 h at 37°C or 4°C. Cells were harvested, washed twice with FACS buffer, and immediately analyzed by flow cytometry.

RNA analysis

Flow cytometry-sorted respective populations of cells were centrifuged and resuspended in 0.5ml TRIzol buffer (Takara). The RNAs for RNA sequencing (RNA-seq) analysis were prepared and sequenced by Wuxi Aptech (Shanghai, China). Briefly, total RNA was extracted using RNeasy protect Mini Kit (Qiagen), the RNA sequencing library was built using the Illumina TruSeq RNA Sample Prep Kit version 2 (Illumina, San Diego, CA), and sequencing was performed on the Illumina HiSeq2500 platform (Illumina). Paired-end RNA-seq analysis obtained 27.5, 27.1, 26.8, and 26.4 million reads from basophils and three different kinds of DCs ($CD11c^+CD49b^+Fc\epsilon RI^+$, $CD11c^+CD49b^-Fc\epsilon RI^+$, and $CD11c^+Fc\epsilon RI^-$), respectively. The reads were then mapped to the mouse genome in Ensembl database using RNA sequence aligner TopHat. On the basis of the aligned result, transcript assembly and abundance estimation were performed with the software Cufflinks. The abundance was quantified in the RPKM unit (numbers of reads per kilobase of exon per million mapped reads). All genes detected were applied to a correlation analysis. Briefly, the Pearson correlation array (P) of four samples was calculated. To generate dendrogram, we used P as distance matrix.

The expression profiles of some selected genes were validated by real-time RT-PCR. Total RNA was extracted from sorted different populations of cells using RNeasy Plus (Takara). RNA was reverse transcribed using PrimeScriptRT reagent Kit. PCRs were performed with SYBR Premix Ex TaqTM (Takara) with primers as listed in the table (Supplemental Table I). Normalization was performed using specific amplification of GAPDH and qPCRs were performed in triplicate for each biological experiment. All relevant quantitative RT-PCR reagents were obtained from Takara. Relative expression levels were calculated using ΔCt method ($Ct_{target} - Ct_{GAPDH}$). Quantification of gene expression levels in each sample was performed by calculating $2^{-\Delta Ct}$. Data are shown as averages from three biological repeats plus SEM.

Statistics

Graphs were generated by GraphPad Prism 5 (GraphPad Software). Statistics were performed as illustrated in relevant figure legends with significance: * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$.

Results

Infiltrated FcεRI-positive cells in the spleen during *S. japonica* infection display higher potency in inducing Th2 cells in vitro than FcεRI-negative cells

A common feature appears that APCs, which can direct Th2 differentiation, are often found among cells positive for FcεRI

including basophils (11–13), mast cells (16, 17), and subset of DCs (2, 18). We thus at first evaluated and compared the Th2-promoting capacities of FcεRI-positive and FcεRI-negative NBNT cells recovered from *S. japonica*-infected spleens to identify the cell populations which can independently function as APCs to promote Th2 response in *S. japonica* infection. *S. japonica* infection in mice characteristically induces Th2 response when infection enters the egg stage, typically around 4 wk following worm invasion, with peak levels of IL-4-producing Th2 cells present at 5 wk postinfection (Supplementary Fig. 1). The number of FcεRI-positive NBNT cells assessed by immunostaining with the mAb recognizing the α subunit of FcεRI (MAR-1), was abruptly elevated at the fifth week postinfection, corresponding to the peak induction of Th2 response (Fig. 1A).

The Th polarization potency by FcεRI-positive NBNT cells and FcεRI-negative NBNT cells were then determined by coculturing the sorted cells with OVA-specific naive $CD4^+$ T lymphocytes obtained from DO11.10 transgenic mice in the presence of OVA_{323–339} peptides. FcεRI-positive cells demonstrated prominent capabilities to induce IL-4-producing Th2 cells. FcεRI-negative cells, in contrast, primarily induced IFN- γ -producing Th1 cells (Fig. 1B). Therefore, the APCs, which can independently initiate Th2 phenotype in *S. japonica* infection, may primarily reside in APCs that are positive for FcεRI. Additional examination found that these FcεRI-positive cells are neither c-kit-positive mast cells nor siglec-F-positive eosinophils (data not show), both of which are known to be recruited to the spleen in large numbers during worm infection (7).

A novel Th2-promoting DC subset ($CD11c^+CD49b^+Fc\epsilon RI^+$) is induced during egg stage in schistosome infection

To further analyze the cell type within FcεRI-positive cells which are able to promote Th2 responses independently, the FcεRI-positive cells obtained from 5 wk infected spleen were further separated using surface markers FcεRI and CD49b for mouse basophils (19) and CD11c for mouse DCs. In addition to basophils ($CD11c^+CD49b^+Fc\epsilon RI^+$), two subsets of CD11c-positive dendritic cells which are also positive for FcεRI are identified (Fig. 2A).

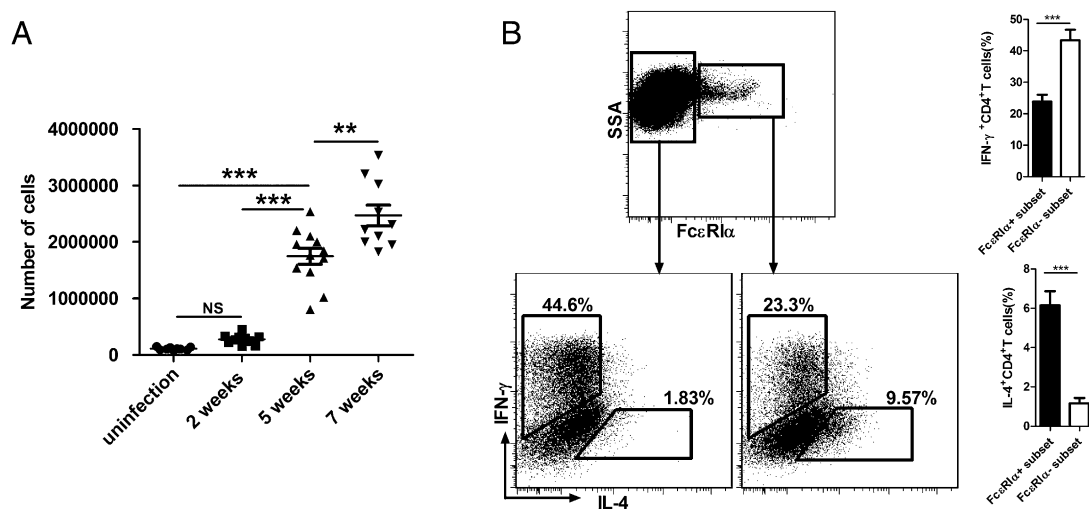


FIGURE 1. FcεRI-positive NBNT cells, which were greatly induced in the spleen during *S. japonica* infection displayed higher potency in promoting Th2 differentiation than FcεRI-negative NBNT cells. (A) The number of FcεRI⁺ cells in the spleen was significantly increased during *S. japonica* infection. (B) Five weeks postinfection, FcεRI⁺ and FcεRI⁻ cells gated on NBNT ($CD3^+B220^-$) cells were flow cytometry-purified from infected spleen (shown in upper left panel) and cocultured with DO11.10 naive $CD4^+$ T cells at 1:2 in the presence of OVA_{323–339}. IFN- γ ⁺CD4⁺ T cells or IL-4⁺CD4⁺ T cells were measured on the sixth day. A representative ICC staining (lower left panel) and the summarized results (right panel) were shown. Bar graphs represented the mean + SEM from three pooled independent experiments with 9–15 mice (A) and 8 mice (B) per group. ** $p < 0.01$, *** $p < 0.001$ by one-way ANOVA with Bonferroni correction (A) or by unpaired t test (B).

One subset is negative for CD49b ($CD11c^+CD49b^-Fc\epsilon RI^+$), which is phenotypically the same as the DC subset reported to be important in Th2 initiation in mice challenged by house dust mite (HDM) allergens (2); the other is a novel subset positive for all three markers examined namely $CD11c^+CD49b^+Fc\epsilon RI^+$ (Fig. 2A).

When these cell populations were examined for their Th polarization activities in vitro, basophils were able to induce Th2 cells as expected (Fig. 2B). More importantly, it was the novel $CD11c^+CD49b^+Fc\epsilon RI^+$ DC subset not the reported $CD11c^+CD49b^-Fc\epsilon RI^+$ DC subset that demonstrated robust Th2-promoting activities

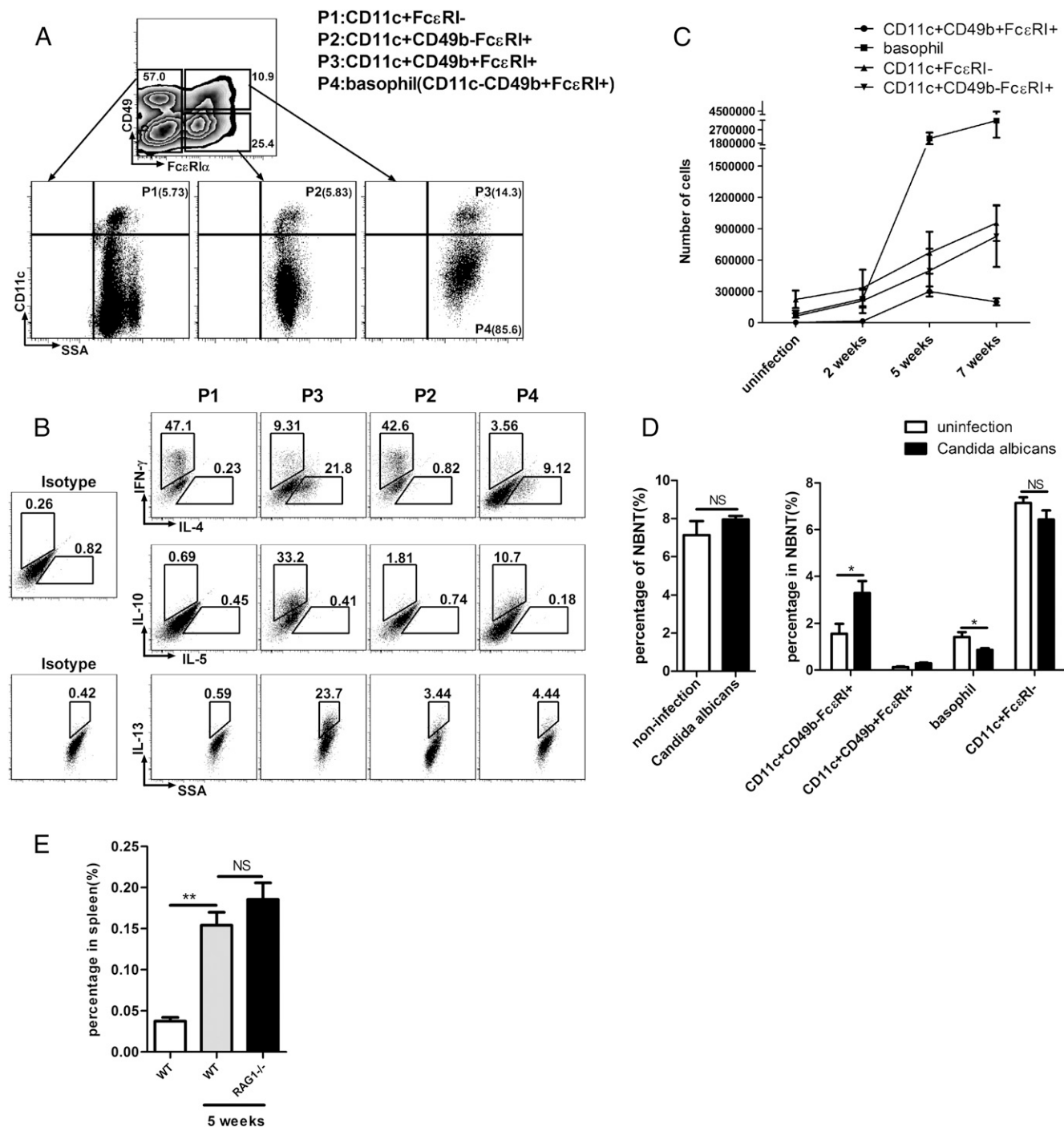


FIGURE 2. A novel Th2-promoting DC subset ($CD11c^+CD49b^+Fc\epsilon RI^+$) was induced during egg stage in *S. japonica* infection. **(A)** Surface expression of CD11c, FcεRI, and CD49b within splenic NBNT cells ($CD3e^-B220^-$) from 5-wk infected mice. The indicated cell subsets included P1 ($CD11c^+Fc\epsilon RI^-$), P2 ($CD11c^+CD49b^-Fc\epsilon RI^+$), P3 ($CD11c^+CD49b^+Fc\epsilon RI^+$), and P4 ($CD11c^-CD49b^+Fc\epsilon RI^+$). **(B)** The four distinct subsets sorted from 5-wk infection mice were cocultured with DO.11.10 naive $CD4^+$ T cells at 1:2 ratio in the presence of OVA₃₂₃₋₃₃₉. Six days later, IL-4-, IL-13-, IL-10-producing Th2 cells and IFN- γ -producing Th1 cells were detected. Dot plots shown were representative for at least five independent experiments. **(C)** Time course on the numbers of relevant cell populations during *S. japonica* infection. Graph shown was mean $\pm$ SEM from three pooled independent experiments with seven to nine mice per time point. **(D)** The mean percentage of NBNT cells in total spleen cells and relevant cell populations in NBNT cells at 6 d after *C. albicans* infection. Bar graphs showed the mean $\pm$ SEM from two pooled independent experiments with five to eight mice per group. **(E)** Percentage of DC2 cells ($CD11c^+CD49b^+Fc\epsilon RI^+$) in total splenocytes in WT or RAG1 $^{-/-}$ mice after 5 wk infection and in uninfected WT mice. Bar graphs represented the mean $\pm$ SEM from five mice in each group. ** $p < 0.01$ by unpaired *t* test.

(Fig. 2B). $CD11c^+CD49b^-Fc\epsilon RI^+$ DC subset as well as splenic conventional DCs identified as $Fc\epsilon RI^-CD11c^{hi}MHC\ class\ II^{hi}$, in contrast, only displayed Th1-promoting activities (Fig. 2B). Thus, in schistosome infection, this novel DC subset likely functions as DC2 to promote Th2 differentiation, whereas conventional DC and $CD11c^+CD49b^-Fc\epsilon RI^+$ DCs function as DC1 to promote Th1 differentiation.

As compared with basophils, this newly identified DC2 subset not only induced IL-4 production by Th2 cells at higher levels, but also induced IL-13-producing and IL-10-producing Th2 cells (Fig. 2B). In addition, IFN- γ -producing Th1 response induced by $CD11c^+CD49b^+Fc\epsilon RI^+$ DC subset was compromised when compared with the Th1 response induced by conventional DCs and $CD11c^+CD49b^-Fc\epsilon RI^+$ DCs. Notably, Th1 response was less compromised if compared with basophils. Therefore, unlike basophils that possesses primarily Th2-promoting activities, this novel $Fc\epsilon RI$ -positive DC2 possesses both Th2-promoting and Th1-promoting activities.

We also investigated the induction of $CD11c^+CD49b^+Fc\epsilon RI^+$ DC2 cells in various infection scenarios. In mice infected with Th2-provoking schistosome infection, the number of DC2 subset reached peak level, which is $\sim 20\%$ of $CD11c^+$ DCs around the fifth week postinfection (Fig. 2C), corresponding well to the occurrence of Th2 cells (Supplemental Fig. 1). In contrast, the number of basophils, conventional DCs and $CD11c^+CD49b^-Fc\epsilon RI^+$ DC1 cells were constantly increased in spleen during infection (Fig. 2C). Furthermore, infection of mice with *C. albicans*, a non-Th2 but Th1/Th17-inducing pathogen (20), no $CD11c^+CD49b^+Fc\epsilon RI^+$ cells were induced within the spleen, and the percentage of $CD11c^+CD49b^-Fc\epsilon RI^+$ DC1 was enhanced (Fig. 2D).

During helminth infection, increased infiltration of NBNT cells into immune organs is considered to be consequences of adaptive immune response especially Th2 response (21). To explore T cell-associated effects on induction of this novel DC2 cells, we exposed $RAG1^{-/-}$ mice to *S. japonica* infection and found similar percentage of $CD11c^+CD49b^+Fc\epsilon RI^+$ DC2 cells present in 5-wk infected spleen comparing with WT mice, although the size and cellularity of the spleen in infected $RAG1^{-/-}$ mice were both markedly decreased (Fig. 2E). Taken together, *S. japonica* infection in mice leads to induction of a novel subset of Th2-promoting DC2 cells with phenotype of $CD11c^+CD49b^+$

$Fc\epsilon RI^+$ via T cell-independent fashion, which may be important in promoting Th2 response in schistosome infection.

CD11c⁺CD49b⁺FcεRI⁺ DC2 cells are IL-4-competent and able to potently promote Th2 differentiation via IL-4-dependent fashion

We then explored whether Th2-skewing abilities of this novel DC2 are IL-4-dependent like that in basophils (13). First, lack of IL-4 did not affect the induction of this novel DC2 population and basophils following schistosome infection (Supplemental Fig. 2A). The Th2-promoting activities, however, were largely abolished in DC2 cells isolated from infected IL-4-deficient mice when compared with the cells isolated from similarly infected WT mice (Fig. 3A). Therefore, like basophils, the Th2-polarization activities by $CD11c^+CD49b^+Fc\epsilon RI^+$ DC2 cells are also IL-4 dependent.

This unexpected finding led us to examine whether this specialized DC2 subset can generate IL-4. The IL-4 contents in $CD11c^+CD49b^+Fc\epsilon RI^+$ DC2 isolated from egg stage-infected spleen were determined by quantitative RT-PCR. A similar amount of IL-4 mRNA was transcribed by DC2 cells as compared with basophils, which was 10- to 20-fold higher than the IL-4 mRNA transcribed by Th1-promoting DC1 (i.e., $CD11c^+CD49b^-Fc\epsilon RI^+$ DCs and $Fc\epsilon RI$ -negative conventional DCs) (Fig. 3B). Notably, the amount of the Th1 polarization cytokine IL-12p40 transcribed by DC2 subset was significantly higher than that by basophils, which may explain the less compromised Th1-promoting activities found in DC2 (Fig. 2B).

CD11c⁺CD49b⁺FcεRI⁺ DC2 has characteristics of both DCs and basophils and possesses stronger Ag-presenting activities than basophils

Morphologically, the $CD11c^+CD49b^+Fc\epsilon RI^+$ DC2 cells as well as $CD11c^+CD49b^-Fc\epsilon RI^+$ DC1 cells resembled splenic conventional DCs in terms of characteristic membrane spikes and lack of indented nuclear morphology (Fig. 4A). Furthermore, like conventional DCs and $CD11c^+CD49b^-Fc\epsilon RI^+$ cells, this newly identified DC2 subset also expressed CD8, whereas basophils were only slightly positive for CD8 (Fig. 4B). When MHC class II and costimulatory molecules including CD80, CD86, and CD40 were examined, this novel DC2 expressed much higher levels of MHC class II than basophils. In addition, higher levels of

FIGURE 3. $CD11c^+CD49b^+Fc\epsilon RI^+$ DC2 cells are IL-4-competent and able to potently promote Th2 differentiation via IL-4-dependent fashion. **(A)** $CD11c^+CD49b^+Fc\epsilon RI^+$ and basophils ($CD11c^-CD49b^+Fc\epsilon RI^+$) sorted from infected spleen of either WT or IL-4 knockout mice were cocultured with DO.11.10 naive $CD4^+$ T cells at 1:2 ratio in the presence of OVA_{323–339} for 6 d. IL-4-producing Th2 cells and IFN- γ -producing Th1 cells were measured. A representative ICC staining (left panel) and the summarized results (right panel) were shown. **(B)** IL-4 and IL-12p40 mRNA expressions in indicated cell populations were measured by real-time RT-PCR, and expression levels were calculated by the value of $2^{-\Delta Ct}$ as shown in *Materials and Methods*. Bar graphs represented the mean $\pm$ SEM from three independent experiments (A and B). For each independent experiment, sorted distinct subsets were pooled from five mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by unpaired *t* test.

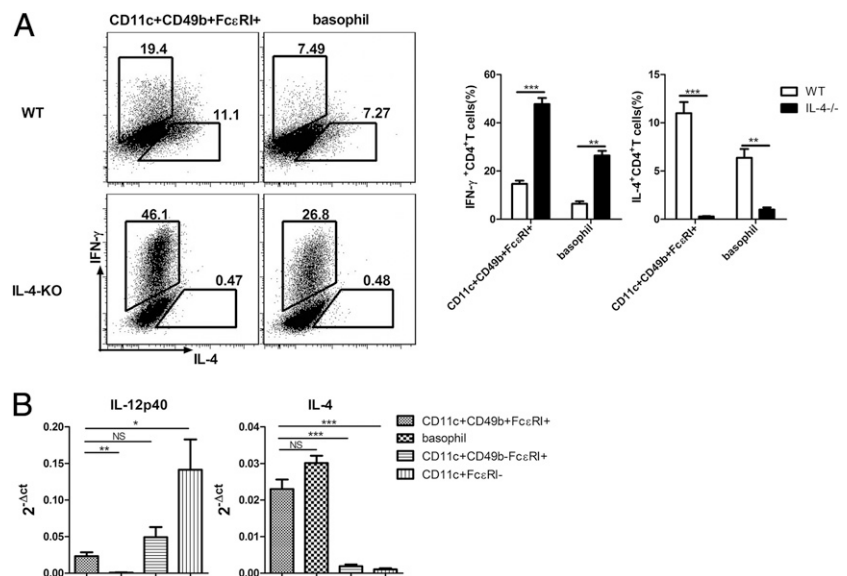
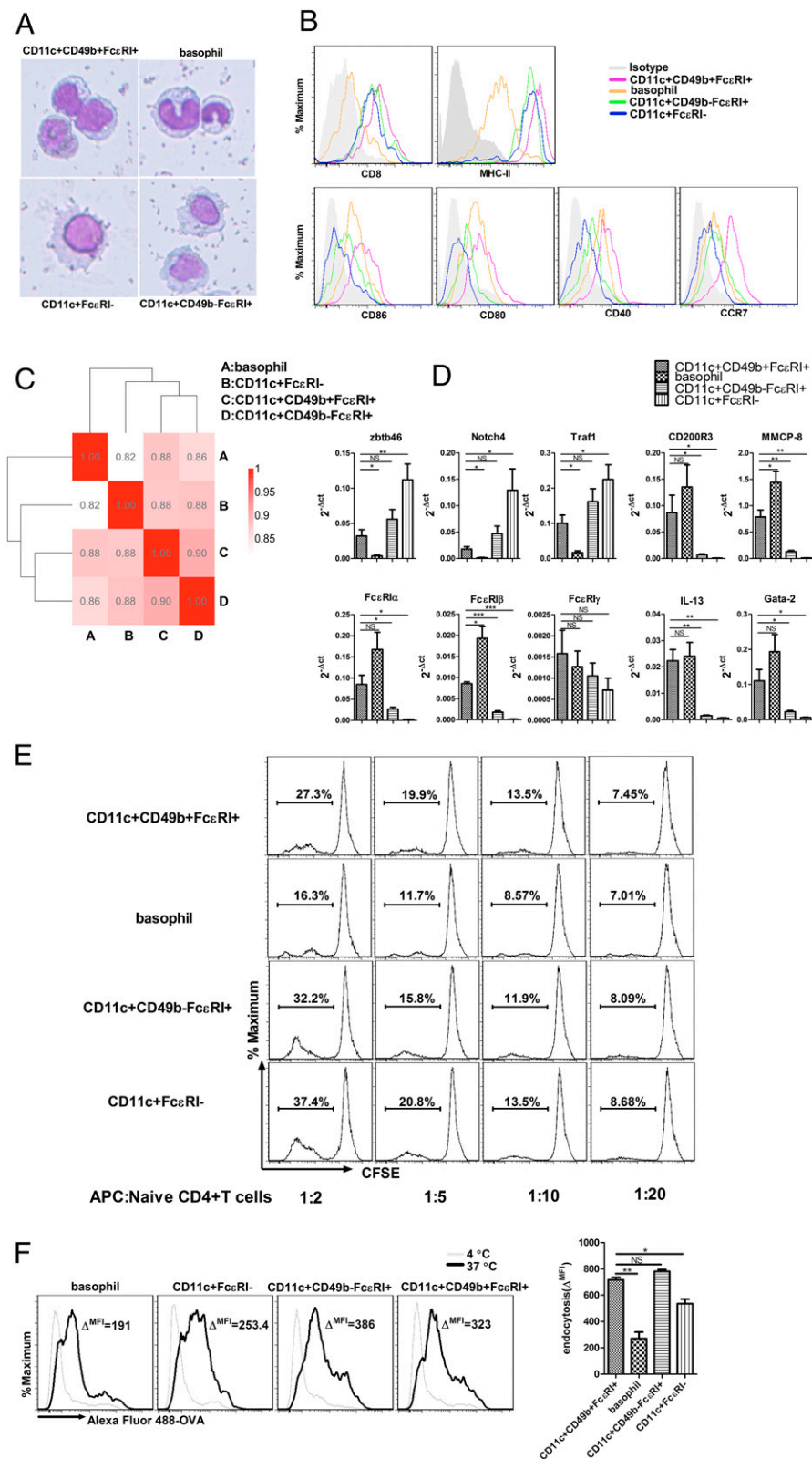


FIGURE 4. CD11c⁺CD49b⁺FcεRI⁺ DC2 has characteristics of both DCs and basophils and possesses stronger Ag-presenting activities than basophils. **(A)** The indicated cell subsets including CD11c⁺FcεRI⁺, CD11c⁺CD49b⁺FcεRI⁺, basophils (CD11c⁺CD49b⁺FcεRI⁺), and CD11c⁺CD49b⁺FcεRI⁺ gated on NBNT (CD3e⁺B220⁺) cells were sorted from spleens of 5-wk infection mice and stained with Giemsa. Images were taken under original magnification $\times 400$. **(B)** Surface expression of MHC class II, CD8, CD86, CD80, CD40, and CCR7 on relevant cell populations gated from splenic NBNT cells. Histograms shown were representative of three independent experiments. **(C)** RNA-seq results of CD11c⁺CD49b⁺FcεRI⁺, basophils (CD11c⁺CD49b⁺FcεRI⁺), CD11c⁺CD49b⁺FcεRI⁺, and CD11c⁺FcεRI⁺ cells were analyzed using R-studio, and Pearson correlation coefficient was displayed. Heat map revealed the Pearson correlation across four samples. Numbers in each square represent correlation between two samples. The square color represents: high correlation (red) or low correlation (white). **(D)** Real-time RT-PCR analysis of mRNA expression of FcεRIα, FcεRIβ, FcεRIγ, GATA-2, MMCP-8, IL-13, CD200R3, zbtb46, Notch4, and TNFR-associated factor 1 in relevant cell populations sorted from spleens of 5-wk infection mice. Expression levels were calculated by the value of $2^{-\Delta Ct}$ as shown in *Materials and Methods*. **(E)** CD11c⁺CD49b⁺FcεRI⁺, basophils (CD11c⁺CD49b⁺FcεRI⁺), and CD11c⁺CD49b⁺FcεRI⁺ cells sorted from the spleen of 5-wk infection mice were cocultured with CFSE-labeled naive CD4⁺T cells derived from DO.11.10 mice in the presence of OVA (40 μg/ml) for 60 h at indicated ratios. Histograms shown are representative of three independent experiments. **(F)** The sorted cells were exposed to Alexa Fluor 488-labeled OVA (40 μg/ml) at 37°C or at 4°C for 5 h. The endocytosis of the sorted cells was assessed by comparing the differences on mean fluorescence intensity (MFI) between 37°C (black curve) and 4°C (gray curve). A representative histogram (left panel) and the summarized results (right panel) are shown. Bar graphs represented the mean $\pm$ SEM from three independent experiments (D and F). For each independent experiment, mRNA or sorted cells were pooled from five mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by unpaired t test.



costimulatory molecules were found on the surface of novel DC2 cells, which may indicate that they are mature DCs (Fig. 4B).

The overall gene transcription profiles among CD11c⁺CD49b⁺FcεRI⁺ cells, CD11c⁺CD49b⁺FcεRI⁺ cells, basophils, and FcεRI-negative conventional DCs were then assessed by RNA-seq. The CD11c⁺CD49b⁺FcεRI⁺ cells showed equal correlation on expressed gene profiles with conventional DCs and with basophils

($r = 0.88$), whereas the correlation between basophils and conventional DC was shown to be the least ($r = 0.82$) (Fig. 4C). This may indicate that this DC2 is a specialized basophil-like dendritic cell. Indeed, as shown in Fig. 4D, CD11c⁺CD49b⁺FcεRI⁺ cells expressed comparable levels on genes that are commonly expressed by resting or activated basophils including all three subunits (α , β , and γ) of FcεRI, GATA-2, MMCP-8, IL-13, and

CD200R3 (22). Meanwhile, this subset also expressed varied but significant higher levels of DC-specific or DC-related molecules (23) like *zbtb46*, *Notch4*, and *TNFR-associated factor 1* than basophils.

Compared with basophils, this novel DC2 stimulated higher levels of proliferation of naive CD4⁺ T cells when supplemented with OVA proteins (Fig. 4E), corresponding to their enhanced capacities to uptake soluble OVA Ags (Fig. 4F) and to higher expression levels of MHC class II and costimulatory molecules (Fig. 4B). Notably, these novel DC2 cells express CCR7, the chemokine receptor involved in homing to T cell zones in periphery lymphoid tissue (Fig. 4B), whereas basophils fail to express. Taken all together, CD11c⁺CD49b⁺FcεRI⁺ DC2 is basophil-like DC with superior Ag-presenting activities.

CD11c⁺CD49b⁺FcεRI⁺ DCs are monocyte-derived inflammatory DCs

Progress made in recent years has indicated that DCs derived from monocytes in different inflammatory scenarios so called inflammatory DCs are in fact the APCs orchestrating immunity both in Th1-associated type I immune response and in Th2-associated type II immune response (24). The novel Th2-promoting DC2 subset identified by us in *S. japonica* infection was found to express surface molecules characteristic for inflammatory DCs including Ly-6C and CD11b (Fig. 5A). In contrast, FcεRI-negative conventional DCs barely expressed any of these molecules (Fig. 5A).

To examine the likely monocyte origin of this IL-4-competent DC2 subset, BM monocytes from GFP-expressing mice were purified based on expression profiles of CD11c⁺FcεRI⁺CD11b⁺

Ly6-C^{hi} (25) and were adoptively transferred into recipient mice either with infection for 5 wk or without infection as control. As shown in Fig. 5B, as early as 2 d posttransfer, GFP-positive cells were recovered from spleen in both infected and in control animals, but infection greatly boosted the differentiation of the transferred monocytes to become CD11c-positive cells. Remarkably, CD11c⁺CD49b⁺FcεRI⁺ DCs was composed of >80% of monocyte-derived CD11c⁺ cells in infected spleen. In contrast, FcεRI-negative CD11c⁺ splenic conventional DCs were hardly found among monocyte-derived CD11c⁺ cells, suggesting that splenic conventional DCs are rarely derived from monocytes even under schistosome infection. Taken together, these results indicate that infection by helminth *S. japonica* at fifth week can promote BM monocytes to differentiate into CD11c⁺CD49b⁺FcεRI⁺ DCs.

IL-3 is capable of inducing Th2-promoting DC2 from BM cells but is not essential in vivo

The striking phenomenon that CD11c⁺CD49b⁺FcεRI⁺ DC2 cells display characteristics of both basophils and conventional DCs by transcriptome analysis (Fig. 4C) prompted us to assess whether IL-3, the hematopoietic cytokines, which are able to differentiate basophils or DC from BM cells (2), can promote the formation of CD11c⁺CD49b⁺FcεRI⁺ DC2 from BM cells in vitro. We found that IL-3 was able to drive the formation of this cell subset from BM cells (Fig. 6A) as well as from BM monocytes (Fig. 6B), and the effect of IL-3 on CD11c⁺CD49b⁺FcεRI⁺ DC2 cells induction was not dependent on IL-4 either (Supplemental Fig. 2B). More significantly, the CD11c⁺CD49b⁺FcεRI⁺

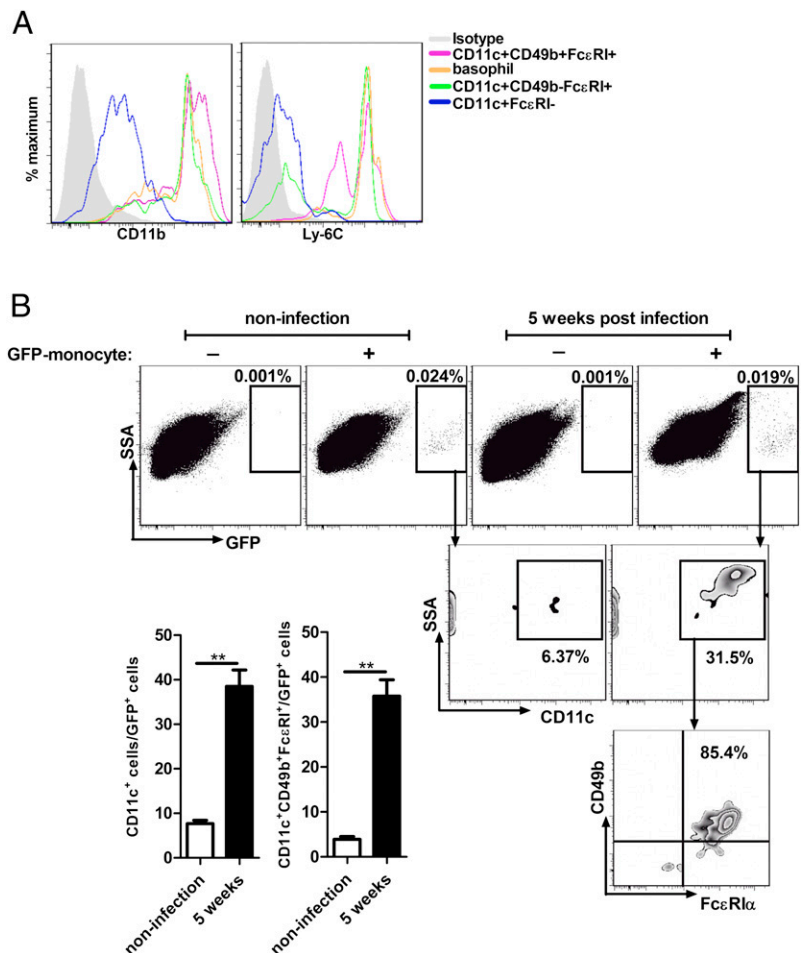


FIGURE 5. CD11c⁺CD49b⁺FcεRI⁺ DCs are monocyte-derived inflammatory DC. (A) Surface expression of Ly-6C and CD11b on CD11c⁺CD49b⁺FcεRI⁺, basophils (CD11c⁺CD49b⁺FcεRI⁺), and CD11c⁺CD49b⁺FcεRI⁻ and CD11c⁺FcεRI⁻ cells, which were gated from splenic NBNT cells from 5-wk infected mice. (B) Monocytes (CD11c⁺FcεRI⁺CD11b⁺Ly6-C^{hi}) were sorted from BM of uninfected GFP mice and were adoptively transferred into uninfected and 5-wk infected mice via tail vein. Two days posttransfer, GFP-positive cells in the spleen were recovered and the expression of CD11c, CD49b, and FcεRI on their surface was monitored. A representative dot-plot (upper panel) and the summarized results (lower panel) are shown. Bar graphs show the mean + SEM from two pooled independent experiments with six to eight mice per group. ***p* < 0.01 by unpaired *t* test.

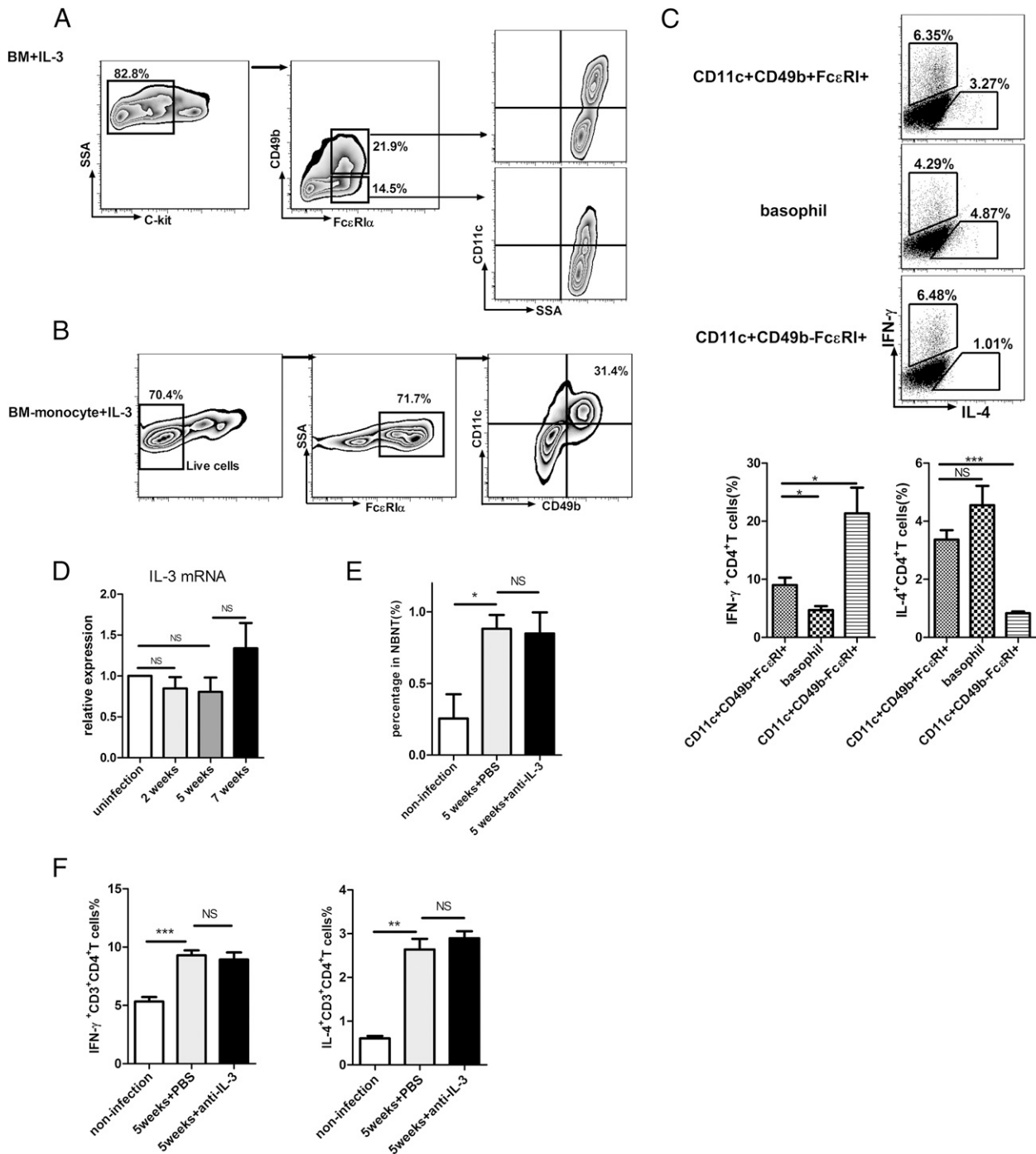


FIGURE 6. IL-3 is capable of inducing Th2-promoting DC2 from BM cells in vitro but is not essential in vivo. WT BM cells were cultured in the presence of mouse IL-3 for 12 d (**A**) and WT BM monocytes were cultured in the presence of mouse IL-3 for 9 d (**B**). Cell subset constitution was analyzed by flow cytometry. (**C**) CD11c⁺CD49b⁺FcεRI⁺, basophils (CD11c⁺CD49b⁺FcεRI⁺), and CD11c⁺CD49b⁺FcεRI⁺ cells generated from IL-3-differentiated BM cultures were cocultured with DO.11.10 naive CD4⁺ T cells. Six days later, IL-4-producing Th2 and IFN-γ-producing Th1 cells were detected by ICC. A representative ICC staining (upper panel) and the summarized results (lower panel) are shown. (**D**) Expression of IL-3 mRNA in spleen postinfection relative to noninfection animals was measured using quantitative RT-PCR. Bar graphs show the mean + SEM from three independent experiments with five mice per group. After administration of anti-mouse IL-3 mAbs, the percentage of DC2 in NBNT (**E**) and IL-4-producing Th2 cells and IFN-γ-producing Th1 cells (**F**) in spleen was examined by flow cytometry. Bar graphs show the mean + SEM from five mice per group. **p* < 0.05, ***p* < 0.01, ****p* < 0.001 by unpaired *t* test.

cells derived from IL-3-differentiated culture were also capable of promoting Th2 cells when they were cocultured with naive CD4⁺ T cells in vitro (Fig. 6C). Thus, IL-3 is able to differentiate BM cells and monocytes into CD11c⁺CD49b⁺FcεRI⁺ DC2.

To explore the in vivo role of IL-3 on induction of DC2 cells in schistosome infection, we assessed changes of IL-3 mRNA ex-

pression in spleen following schistosome infection. No upregulation of IL-3 mRNA was found before the fifth week of infection, the time point when DC2 cells are most prominent (Fig. 6D). Furthermore, administration of mAb against mouse IL-3 in infected animals before appearance of CD11c⁺CD49b⁺FcεRI⁺ DC2 cells failed to show any abrogating effects on induction of DC2 cells or

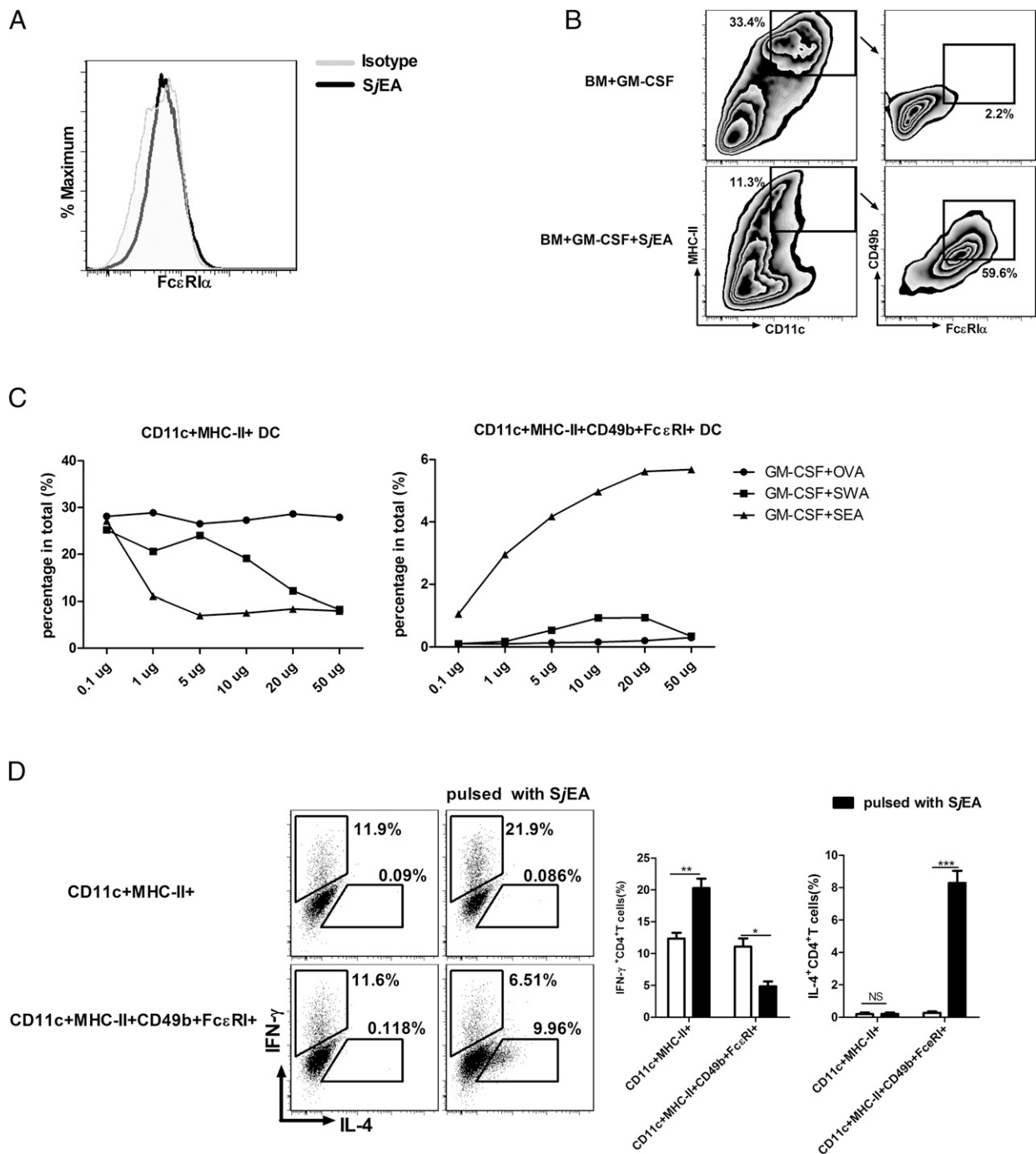


FIGURE 7. Soluble egg Ags are able to drive the differentiation of BM cells into Th2-promoting CD11c⁺CD49b⁺FcεRI⁺ DC2. **(A)** Expression of FcεRI on GM-CSF induced BMDC (CD11c⁺MHC-II⁺FcεRI⁺) after being stimulated with SjEA for 24 h. **(B)** WT BM cells were cultured in the addition of mouse GM-CSF (25 ng/ml) or GM-CSF (25 ng/ml) plus SjEA (10 μg/ml) for 5 d. Cell subset constitution was analyzed by flow cytometry. **(C)** WT BM cells were cultured in the addition of mouse GM-CSF (25 ng/ml) plus different concentration of SjEA, SWA, or OVA for 5 d. The percentage of CD11c⁺MHC-II⁺ DC and CD11c⁺MHC-II⁺CD49b⁺FcεRI⁺ DC was analyzed by flow cytometry. **(D)** CD11c⁺MHC-II⁺ DC induced by GM-CSF only and CD11c⁺MHC-II⁺CD49b⁺FcεRI⁺ DC induced by GM-CSF plus SjEA were pulsed with SjEA (50 μg/ml) overnight and washed with RPMI 1640 medium, then cocultured with naive CD4⁺ T cells (DO.11.10) for 6 d. IL-4-producing Th2 and IFN-γ-producing Th1 cells were detected by ICC. A representative ICC staining (left panel) and summarized results (right panel) are shown. Bar graphs represent the mean + SEM from three experiments. **p* < 0.05, ****p* < 0.001, ***p* < 0.01 by unpaired *t* test. Results shown (A–C) are also representative for three independent experiments.

Th2 cells in spleen comparing to control mice (Fig. 6E, 6F). This is in accordance with previously published report that IL-3 deficiency does not affect Th2-type immune responses in helminth infection (26). Collectively, these results indicate that IL-3 is only sufficient but not necessary for DC2 induction by *S. japonica* infection.

Soluble egg Ags are able to drive the differentiation of BM cells into Th2-promoting CD11c⁺CD49b⁺FcεRI⁺ DC2

The correlation between the appearance of specialized DC2 cells and egg stage-associated Th2 response in schistosome infection led us to speculate whether SjEA can facilitate the induction of CD11c⁺CD49b⁺FcεRI⁺ DC2. When BM-derived DCs (BMDCs) obtained

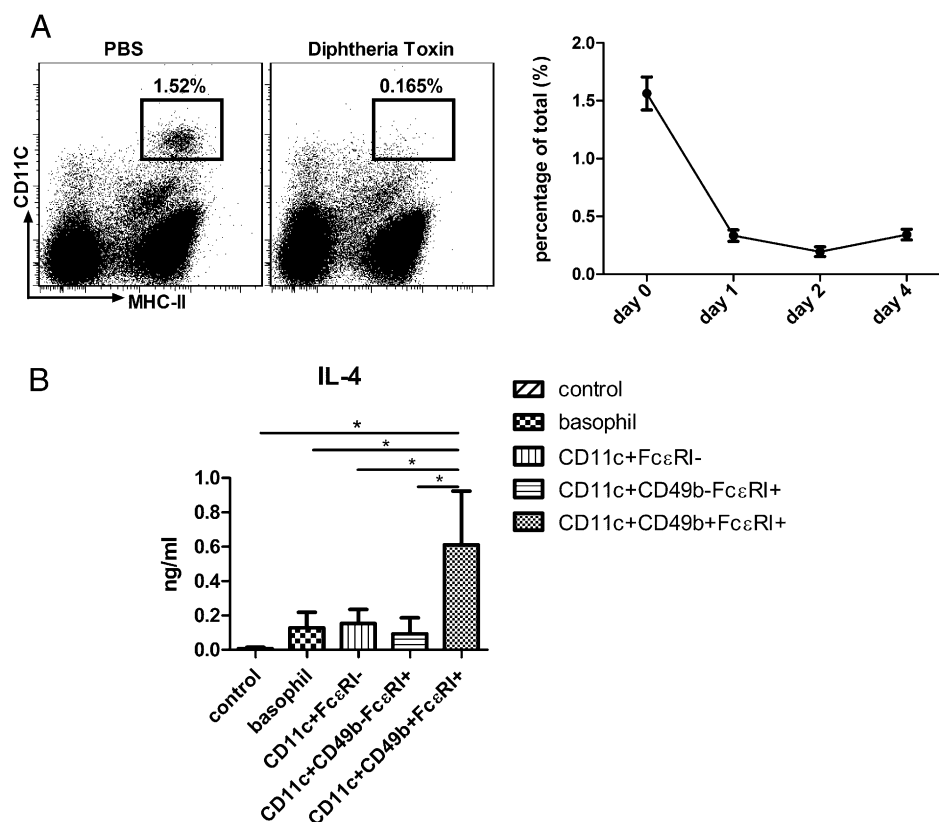


FIGURE 8. CD11c⁺CD49b⁺FceRI⁺ DC2 is sufficient to drive Th2 cells in vivo. **(A)** DT treatment effectively depleted splenic MHC-II⁺CD11c⁺ DC in CD11c-DTR mice, and depletion lasted at least for 4 d. A representative dot plot shows the depletion of spleen DC (CD11c⁺MHC-II⁺) at 1 d (left panel), and data (right panel) show the depletion of spleen DC (CD11c⁺MHC-II⁺) at days 1, 2, and 4 post-DT injection. Data shown was mean of three mice per time point. **(B)** CD11c⁺CD49b⁺FceRI⁺, basophils, and CD11c⁺CD49b⁺FceRI⁺ and CD11c⁺FceRI⁻ cells sorted from the spleen of 5-wk infection mice were loaded with OVA₃₂₃₋₃₃₉ and injected i.v. into CD11c-DTR mice, which was treated with DT and transferred with naive CD4⁺ T cells (DO.11.10) 1 d before. Control mice were only treated with DT and transferred with naive CD4⁺ T cells without adoptive transfer of additional APCs. Five days after APC transfer, splenocytes were stimulated with OVA₃₂₃₋₃₃₉ (10 µg/ml) for 4 d in vitro, concentrations of IL-4 in supernatants were measured using ELISA. Bar graphs shown are the mean + SEM from two pooled independent experiments with six to eight mice per group. **p* < 0.05 by unpaired *t* test.

from 5 d GM-CSF treatment were cultured with SjEA, the obtained FceRI-negative DCs failed to be switched to FceRI-positive DCs by SjEA (Fig. 7A). However, if SjEA was added simultaneously with GM-CSF into BM cell cultures, SjEA was able to steer majority of the induced CD11c⁺MHC-II⁺ DCs into CD11c⁺CD49b⁺FceRI⁺ DC2 at a dose-dependent fashion, although some degree of inhibition of dendritic cell differentiation was noticed in the presence of SjEA (Fig. 7B, 7C). Interestingly, addition of worm Ags isolated from male worms or irrelevant protein OVA failed to show any effects (Fig. 7C). Therefore, egg Ags can specifically facilitate differentiation of FceRI-positive DCs from BM cells. When egg Ags promoted DC2 cells were examined for their Th2 polarization activities using DC-T coculturing system, IL-4-producing Th2 cells were also formed (Fig. 7D). Notably, no Th2 cells were induced if BMDCs were pulsed with egg Ags before DC-T coculture (Fig. 7D). It indicated that, on the contrary to egg Ag from *S. mansoni* (SmEA) (27), SjEA is unable to condition conventional DCs to possess Th2-promoting activities. Thus, SjEA exert their Th2-inducing effects likely through induction of specialized DC2 from BM precursor cells but not through change of functionality of conventional DCs.

CD11c⁺CD49b⁺FceRI⁺ DC2 is sufficient to drive Th2 cells in vivo

Last, we determined the Th2 polarization activities of the novel DC2 in vivo. DC2 cells as well as basophils and DC1 subsets were isolated from 5-wk infected mice. After being loaded with OVA

peptides, these cell populations were adoptively transferred to CD11c-DTR mice in which endogenous CD11c⁺MHC-II⁺ DCs had been largely depleted by DT injection (Fig. 8A) (28), and naive DO11.10 CD4 T cells had been transferred 1 d ahead. Spleen cells were collected 5 d after basophils or different DC subsets transfer and were restimulated in vitro for 4 d with OVA₃₂₃₋₃₃₉ peptides. As shown in Fig. 8B, only splenocytes obtained from animals transferred with DC2 cells generated detectable IL-4 upon restimulation. Therefore, CD11c⁺CD49b⁺FceRI⁺ DC2 subset is sufficient to position and promote Th2 response in vivo, whereas basophils fail to. Higher levels of MHC class II and CCR7 expressed and better soluble Ag uptake activities may all confer CD11c⁺CD49b⁺FceRI⁺ DC2 cells with superior Ag-presenting activities in vivo.

Discussion

Our understanding toward initiation of Th2 immune response via DCs differs from initiation of Th1 and Th17 immune response in that there is no clear answer on the nature of polarization force used by DCs to induce Th2 cells. DCs produce Th1-inducing and Th17-inducing cytokines like IL-12 or IL-6 and IL-23 while they present Ags and polarize naive CD4⁺ T cells into specialized Th1 or Th17 subsets, whereas DCs do not appear to produce Th2-inducing cytokine IL-4. Therefore, “default” model has been proposed in which Th2 polarization is thought to be resulted from lack of Th1-polarizing cytokine IL-12p70 production. Th2 polarization in-

duced by DCs conditioned with *Porphyromonas gingivalis* LPS, cholera toxin, SmEA, and thymic stromal lymphopoietin has all been reported to be correlated with reduced IL-12p70 production (29). However, it is hard to envision that this mode is important in in vivo scenarios because most Th2 response-associated pathogens also contain Th1-inducing components. Thus, it is impossible to exclude that DCs instruct Th2 differentiation depending on generation of specific signals. The coexistence of both Th1 and Th2 cells stimulated by DCs conditioned with Th2-promoting Ags SmEA together with Th1-promoting Ags supports this notion (30). The DC2 demonstrated in this report also supports the instruction model, since both Th2- and Th1-promoting activities are possessed by DC2 reported in this study.

No unified mechanisms are obtained regarding the nature of signals used by DCs to instruct Th2 differentiation. OX40L expressed on thymic stromal lymphopoietin-conditioned DCs determines the induction of Th2 cells (31). Notch ligand jagged-2 seems to allow DCs to promote Th2 responses via IL-4-independent mechanism, and the expression of jagged-2 is induced on DCs by signals that predispose to Th2 responses, such as PGE2 and cholera toxin (32). Contrary to this, Th2 response induced by SmEA-conditioned DCs is unaffected by jagged-2 (33). Our work demonstrated that some specialized DCs can produce instruction signal IL-4 to induce Th2 cells. It is thus possible that both IL-4-dependent and IL-4-independent mechanisms can be attributable for induction of Th2 differentiation by DCs, depending on the nature of microbial or environmental Th2-promoting Ags.

In addition to modify the function of conventional DCs, Th2-inducing microbes or allergens have evolved alternative strategies to achieve DC-directed Th2 response via induction of specialized DC subsets. For example, monocyte-derived FcεRI-positive inflammatory DC is important for Th2 response in mite allergen challenges in mice (2). Non monocyte-derived resident CD301b-positive dermal DCs are critical in initiation of Th2 cell responses in alum- and papain-induced Th2 response when Ags are given locally (34). A unique IRF-4-dependent DC subset is induced under GM-CSF treatment, which can specifically promote Th2 response (35). In this study, we found that infection by *S. japonica* promote the formation of specialized DC2 subset from bone marrow precursor cells, likely through egg Ags, which facilitate Th2 polarization. More significantly, a T cell-independent mechanism is demonstrated for the induction of DC2 subset because DC2 cells were normally induced in *S. japonica*-infected RAG1^{-/-} mice. Therefore, our study provides evidence that induction of DC2 subset can occur ahead of and independent of adaptive immune response, which indicates an important role played by specially differentiated DC2 in promotion of Th2 differentiation.

The Th2-promoting DC2 subset identified by Hammad et al. (2) using HDM inhalation model and the DC2 subset described by us are phenotypically similar because both DCs are remarkably FcεRI positive. The obvious difference on immunophenotype observed is the positivity of CD49b in our DCs. Recognized by well known Ab DX5 originally for NK cells, CD49b was coincidentally used for identification of basophils in mice (19). This integrin molecule has not been shown to be associated in any way with induction of Th2 response; therefore, CD49b is not likely to be responsible for Th2-promoting activities of DC2 cells presented in this study. More significant differences reside in demonstrated IL-4 competence together with IL-4-dependent mechanisms on induction of Th2 cells by our DC2. In addition, Th2 phenotypes induced by our DC2 and infiltration sites of our DC2 are also different. Unlike our DC2 cells, which can promote both IL-4- and IL-13-producing Th2 cells, the DC2 described

by Hammad et al. (2) mainly promotes IL-13-producing Th2 cells. Furthermore, DC2 presented in this work are mainly found in spleen and liver but not in mesenteric lymph nodes (Supplemental Fig. 3) in *S. japonica*-infected animals, which is in contrast to the DC2 reported by Hammad et al. (2), which can be recovered from lung draining lymph nodes after HDM inhalation in animals.

IgE is often a detrimental factor in determination of Th2-promoting activities owned by FcεRI-positive DCs. Expression of FcεRI on dendritic cells via genetic modification is sufficient to allow transfected DCs to promote Th2 response and associated pathologies via IgE-dependent mechanism (18, 36). DC2 cells found in this study were also loaded with IgE (data not show) probably because of they were isolated from IgE-enriched schistosome infected animals. It is hard to clarify whether the IL-4-dependent Th2-promoting activities shown on these novel DC2 cells are resulted from activation of FcεRI by IgE. However, the fact that IL-3-induced or GM-CSF plus S/EA-induced FcεRI-positive DC2 cells are able to promote Th2 cells in vitro without addition of IgE supports the notion that presence of FcεRI along with its activation by IgE is not a prerequisite for Th2 promotion by our DC2 cells. Furthermore, FcεRI-deficient mice infected with *S. masoni* did not demonstrate any impairment in Th2 responses (26). It may be possible that the initial induction of Th2 cells by FcεRI-positive DCs is independent of the activities of FcεRI, but further advancement and maintenance of Th2 cells can be potentiated by regulating activities of FcεRI via IgE.

In summary, the data presented in this work demonstrate for the first time, to our knowledge, that a novel monocyte-derived inflammatory DC2 subset with phenotype of CD11c⁺CD49b⁺FcεRI⁺ is induced during egg stage of *S. japonica* infection. This specialized DC is able to generate IL-4 and promote Th2 differentiation both in vitro and in vivo via IL-4-dependent fashion. Our work together with others suggests that cells positive for FcεRI may be appropriate target cell populations to control Th2 response-associated diseases.

Acknowledgments

We thank Kunshan Zhang from Stem Cell Translational Research Center of Tongji Hospital, Tongji University School of Medicine, for his assistance on analysis of Pearson correlation using R-studio.

Disclosures

The authors have no financial conflicts of interest.

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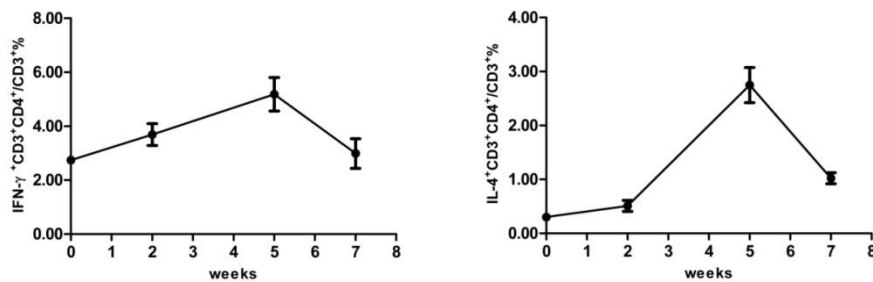
1 **Supplementary Table 1. Primers used in this study**

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	Forward primer (5'--3')	Reverse primer (5'--3')	mRNA ID
IL-4	GAAGGGACGCCATGCACGGA	GCTCACTCTCTGTGGTGTCTTCGT	NM_021283.2
IL-12p40	TGGGAGTACCTGATCCTG	AGGAACGCACCTTTCTGGTT	NM_008352.2
zbtb46	AGCAGCCGAGACTCAAATGTA	TTCCCTTCTCTGGGGTGAGGG	NM_027656.2;NM_028125.3
Notch4	CTCTGTCCCCCAGGTTTCAC	CCCGGGCTTCACATTCATCT	NM_010929.2
Traf1	CTTCAAGGGGAGCCACAAT	GTGCAGACCCTAGGTTGGAG	NM_009421.3
CD200R3	GATTGAGATTACTCCAGTGTCT	CTGATCTGCTGCAACTCTGCT	NM_001128133.1
MMCP-8	AACGCTGAAGGAGGGGAAATC	CCACCACAGCGATAGACTGA	NM_008572.1
FcεRIα	ATTGTGAGTGCCACCGTTCA	GAAGGAGCAGCCAATCTTGC	NM_010184.2
FcεRIβ	GGGTGCAGTGCTGTTTGT	TCTGTGGTGAAAGAAGCCACA	NM_001276328.1
FcεRIγ	CTGTCTACACGGGCCTGAAC	AAAGAATGCAGCCAAGCACG	NM_010185.4
IL-13	ACACAAGACCAGACTCCCCT	CTCTGGGTCTGTAGATGGC	NM_008355.3
GATA2	TGCACAATGTTAACAGGCCAC	CCTCGAAACATTCAGCCCCCT	NM_008090.5
GAPDH	ATGACATCAAGAAGGTGGTG	CATACCAGGAAATGAGCTTG	NM_001289726.1;NM_008084.3
IL-3	TGGAGGACCAGAACGAGACA	TTTGAGTTCAGGTTCTGGGAGC	NM_010556.4

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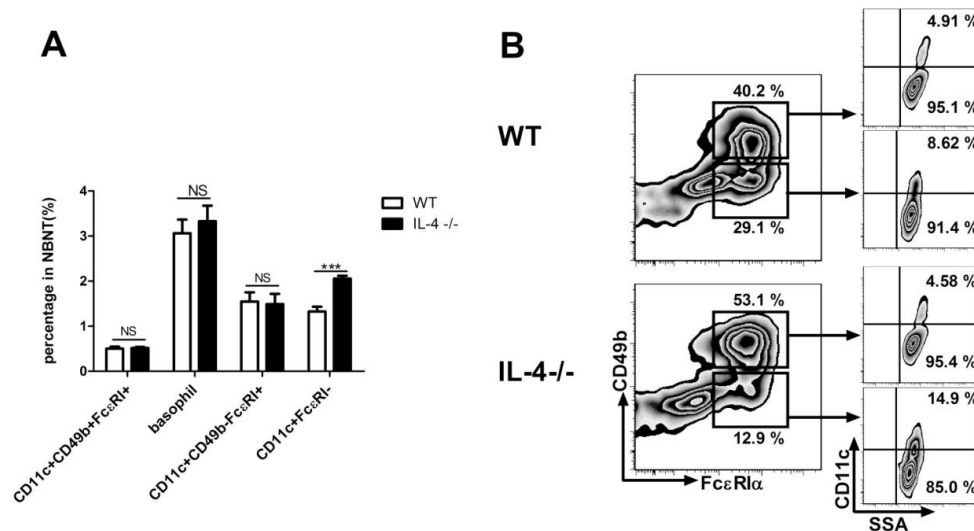
6 **Supplementary Figure 1. Time course of Th1/Th2 cells in the spleen during**

7 ***Schistosoma japonica* infection. IL-4⁺CD4⁺ T cells and IFN-γ⁺CD4⁺ T cells in the**

8 spleen were detected before and 2 weeks, 5 weeks, 7 weeks after *Schistosoma*

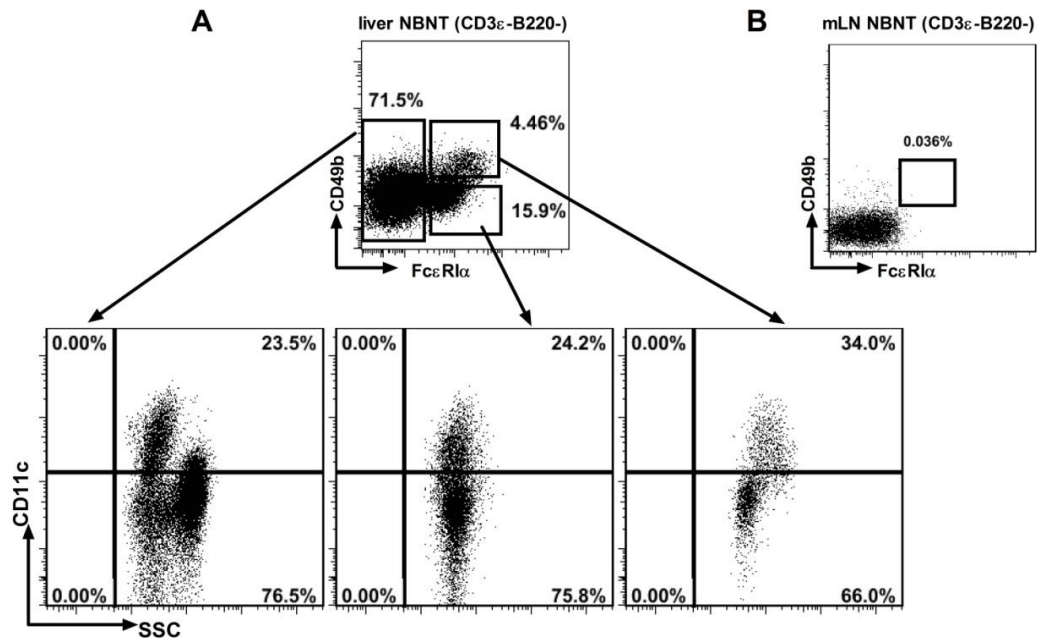
9 *japonica* infection. Graphs show the mean ± SEM from three pooled independent

10 experiments with 15 mice per group.



Supplementary Figure 2. The induction of the CD11c⁺CD49b⁺FcεRI⁺ cells by *Schistosoma japonica* infection and IL-3 was not dependent on IL-4. (A) 5 weeks post infection, the percentage of relative cell populations in NBNT cells of infected spleen were measured in WT and in IL-4-deficient mice. (B) Bone marrow of WT and IL-4-deficient mice were cultured in the presence of mouse IL-3 for 12 days, then the percentage of CD11c⁺CD49b⁺FcεRI⁺, basophils and CD11c⁺CD49b⁻FcεRI⁺ was measured. Zebra gate on c-kit⁻ cells are representative of 3 independent experiments. Bar graphs show the mean + SEM from two pooled experiments with 10 mice per group. Significance was analyzed by unpaired t-test. *** P<0.001, NS means no significant difference.

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32 **Supplementary Figure 3.** CD11c⁺CD49b⁺Fc ϵ RI α ⁺ cells were present in liver but
33 **not in mesenteric lymph nodes in mice infected with *Schistosoma japonica*.** 5
34 weeks post infection, liver leukocytes (A) and mesenteric lymph node cells (B) were
35 isolated and stained with fixable viability dye eFluor 780 together with FITC
36 conjugated anti-mouse CD49b, PerCP/Cy5.5 conjugated anti-mouse CD11c, APC
37 conjugated anti-mouse Fc ϵ RI α and PE conjugated anti-mouse CD3 ϵ and B220, Cells
38 shown were gated on non-B and non-T (NBNT, CD3 ϵ ⁻B220⁻).