Induction of Enhanced Immunity to Intestinal Nematodes Using IL-9-Producing Dendritic Cells

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Dendritic cells can be considered natural adjuvants and are able to act as cellular vaccines to protect against disease. Adoptive transfer of Ag-pulsed bone marrow-derived dendritic cells (BMDCs) enhanced expulsion of the intestinal nematode, *Trichinella spiralis*, from the small intestine. IL-9 is a critical cytokine in protective immunity to intestinal nematode infection and is believed to enhance Th2 immune responses. Deriving dendritic cells from an IL-9 transgenic (IL-9t) mouse has enabled a detailed investigation of the importance of IL-9 during Ag presentation. Indeed, IL-9t dendritic cells significantly enhanced T cell proliferation and Th2 responses and, after adoptive transfer, enhanced parasite-specific IgG1 and intestinal mastocytosis in vivo, leading to accelerated expulsion of adult worms from the intestine. Overall, this paper demonstrates that dendritic cell vaccination can be used to successfully protect the host against intestinal nematode infection and suggests that IL-9 can act as a potent type 2 adjuvant during Ag presentation and the early stages of Th2 activation.

Materials and Methods

**Animals and infection**

Six- to 8-wk-old male FVB mice were purchased from Harlan-Olac. IL-9 transgenic (IL-9t) mice were a gift from Drs. J. Van Snick and J. C. Renaud (Ludwig Institute for Cancer Research, Brussels, Belgium) (12). All experiments were performed under the regulations of the Home Office Scientific Procedures Act (1986).

Infection, maintenance, and recovery of *T. spiralis* were conducted as previously described (13). Experimental mice were infected with 200 *T. spiralis* larvae by oral gavage, and the numbers of adult worms in the small intestine were assessed at various time points postinfection (p.i.).

**Generation of bone marrow-derived DCs (BMDCs)**

BMDCs were established from the femurs of male mice. Briefly, bone marrow cells were flushed out and washed with complete medium before RBC lysis and subsequent washing. BMDCs were generated using a modified previously described method (14). Briefly, BM were plated at 6 × 10⁵ cells/ml with 80 ng/ml GM-CSF (grown in-house) in six-well plates (Corning Costar) and incubated for 3 days at 37°C. An additional 3 ml of complete medium with 80 ng/ml GM-CSF was added on day 3 of culture. Nonadherent cells were collected on day 10 by gently swirling the plates and pipetting off the supernatant. Cell purity was assessed by flow cytometry with hamster anti-mouse CD11c, rat anti-mouse CD80, rat anti-mouse CD86, and rat anti-mouse MHC class II (BD Pharmingen). In some experiments BMDC cultures were purified to 95% CD11c⁺ cells using Miltenyi Biotec CD11c microbeads as described in the manufacturer's protocol.

**Preparation of BMDCs for in vitro and in vivo experiments**

BMDCs were resuspended to 2 × 10⁶ cells/ml in complete medium and stimulated with or without 50 μg/ml *T. spiralis* Ag or 10 μg/ml LPS at 37°C for 8 h for injection or for 24 h to assess phenotype and cytokine production.
secretion. Before injection, cells were washed three times in sterile PBS. Recipient mice received 5 × 10^6 cells/ml i.v. 15 days before infection. Recipients of both pulsed and unpulsed cells were tail bled on day 14 after immunization to determine serum levels of parasite-specific IgG1 and IgG2a. BMDCs were also injected s.c. into the heel of the hind limbs 6 days before removal of popliteal lymph nodes.

**Stimulation of naive T cells with Ag-pulsed DCs**

Single-cell suspensions were created from naive MLN using a 100-μm pore size sterile sieve. Cells were washed before RBC lysis and subsequent washing. Cells were resuspended at 2 × 10^5 cells/ml in wash medium with the addition of 10 μg/ml rat anti-mouse CD8 (clone YTS191) and 10 μg/ml rat anti-mouse B220 (clone RA3-6B2) and were incubated on ice for 45 min. Meanwhile, goat anti-rat IgG magnetic beads (PerSeptive Biosystems) were washed twice in cold wash medium and resuspended to 1 ml for every 2 × 10^6 cells. The cell suspension was washed, resuspended in 1 ml of beads for every 2 × 10^6 cells, and incubated at 4°C with rolling for 30 min. Ab-bound cells were removed by attaching the tubes to a magnet. The unbound cells were collected and resuspended at 1 × 10^6 cells/ml in sterile PBS. Purity was assessed by counterstaining the cells with rat anti-mouse CD4.

**Staining of CD4^+ T cells with CFSE**

Purified CD4^+ T cells were incubated at 37°C for 10 min before addition of 5 μM/ml CFSE. Cells were stained at 37°C for 15 min. Unbound CFSE was neutralized by addition of 100 μl of heat-inactivated, European Economic Community origin FCS to every 1 ml of cells. Cells were washed twice and resuspended in complete medium at 2.5 × 10^6 cells/ml.

**Stimulation of CD4^+ T Cells with BMDCs**

CD4^+ T cells were stimulated with medium alone, 50 μg/ml anti-IL-4R Ab (clone M1) was added to all wells. Cell supernatants were harvested after 24 h and stored at −80°C before analysis.

**Lymph node cell restimulations**

Single-cell suspensions were prepared from the MLN and resuspended in complete medium to a final concentration of 5 × 10^6 cells/ml. One-milliliter cultures were set up in 24-well tissue culture plates (Corning Costar). Cells were stimulated with medium alone, 50 μg/ml T. spiralis Ag as previously described. Unpulsed or pulsed BMDCs (5 × 10^5) were combined with 2.5 × 10^5 CD4^+ T cells. Cells were incubated at 37°C in 48-well plates for 48 h before addition of 20 U/ml recombinant murine IL-2 (rmIL-2) for an additional 72 h. The resulting cell cultures were transferred to a 48-well plate, previously coated overnight with 3 μg/ml plate-bound anti-CD3 (clone 145-2C11). Cells were counterstained with 1 μg/ml anti-CD4 (clone RM4-5) and analyzed for proliferation by flow cytometry 72 h later.

**Mast cell enumeration**

To quantify the level of mastocytosis within the small intestine, 5-mm snips of the small intestine were taken, 100 mm from the stomach, and fixed in Carnoy’s solution before embedding in paraffin wax. Sections were stained with toluidine blue, and mast cells were enumerated by counting the number of mast cells in 20 villus crypts in three sections per animal. An average value was taken to give the mean number of mast cells per 20 villus crypt units.

**Results**

IL-9t BMDCs secrete high levels of IL-12 and IL-9, but fail to express CD80 upon Ag stimulation

DCs were generated from the bone marrow of wild-type (WT) and IL-9t mice and phenotypically analyzed by flow cytometry (Fig. 1, A and B). Unpulsed WT and IL-9t cells were CD8α^+ , CD11c^+ , CD80^− , CD86^lo , and MHC class II^lo-high^, respectively. Routinely, the IL-9t population was 70 ± 10% CD11c^+ . After an 8-h antigenic pulse with *T. spiralis* Ag, both WT and IL-9t DCs up-regulated MHC class II (WT cells, from 50 ± 5 to 70 ± 5%; IL-9t cells, from 40 ± 10 to 73 ± 6%) to a similar level. WT DCs up-regulated the expression of both CD80 and CD86. In contrast, IL-9t DCs up-regulated surface expression of CD86 alone.

The secretion of IL-4, IL-9, and IL-12 by BMDCs was also assessed in response to *T. spiralis* Ag or LPS. WT and IL-9t BMDCs both secreted IL-12 upon stimulation with *T. spiralis* Ag and LPS; however, IL-9t BMDCs secreted significantly higher levels of IL-12 than WT BMDCs (Fig. 1C). Only IL-9t BMDCs secreted detectable levels of IL-9 (Fig. 1D); IL-4 remained undetectable (data not shown) in both cell types.

IL-9 secretion by DCs enhances proliferation of naive WT CD4^+ T cells in a type 2-dependent manner

To investigate whether IL-9 secreted from transgenic BMDCs could enhance Th2-mediated immune responses, in vitro assays were devised to examine the initial stages of T cell priming. Initial experiments focused on the proliferation of naive CD4^+ T cells in response to rmIL-9 (Fig. 2, A and B). It can be clearly seen that proliferation was enhanced and accelerated in those cultures with additional rmIL-9. To establish the response to IL-9 secreted by BMDCs, CD4^+ T cells were purified from naive WT MLN and subjected to stimulation with pulsed WT or IL-9t BMDCs (Fig. 2, C–F). Proliferation of T cells only occurred during the presentation of *T. spiralis* Ag. Moreover, CD4^+ T cells within the IL-9t BMDC cultures not only showed enhanced proliferative responses (Fig. 2, G and H), but underwent additional cell divisions compared with WT BMDC cultures (Fig. 2, G and I). LPS was used to assess whether this response was dependent on the type of Ag used to prime the cells. As with *T. spiralis*-pulsed BMDC cultures, LPS presentation also induced the proliferation of naive T cells (Fig. 2, E and F). In WT BMDC cultures, the pattern of proliferation was similar regardless of the type of antigenic stimulation. In IL-9t BMDC cultures, LPS-pulsed BMDCs induced a higher percentage of T cells to proliferate compared with *T. spiralis*-pulsed BMDCs; however, these cells underwent fewer cell divisions (Fig. 2, G, H, and J).

To investigate the effects of IL-9 secretion on the T cell response, the levels of cytokines secreted into the supernatant by the cells were assessed (Fig. 3). Ag-pulsed IL-9t BMDC cultures contained higher levels of IL-4, IL-5, IL-9, and IL-13 than those cultures containing Ag-pulsed WT BMDCs. Furthermore, the cultures containing *T. spiralis*-pulsed BMDCs contained reduced levels of IFN-γ compared with those cultures with LPS-pulsed BMDCs.

IL-9t BMDCs induce a strong Th2-mediated immune response within the draining lymph nodes

To establish whether IL-9 production by the BMDCs in vivo can induce the potent Th2 responses seen in the in vitro experiments, Ag-pulsed BMDCs were injected s.c. into the heel of naive WT mice.
mice. The popliteal and inguinal lymph nodes (as a control) were removed 6 days later and restimulated with parasite Ag. The most striking difference observed between the lymph nodes from the naive animals and those that received BMDCs was the dramatic increase in IL-4 secretion within both inguinal and popliteal lymph nodes, with popliteal cells secreting elevated levels (Fig. 4). This IL-4 secretion was enhanced within cultures from IL-9t BMDC recipients. The only cultures with detectable levels of IL-9 were restimulated popliteal lymph nodes from pulsed IL-9t BMDC recipients. Both WT and IL-9t BMDC recipients demonstrated enhanced IFN-γ levels in the popliteal lymph nodes, with higher levels in those receiving WT BMDC. IL-12 secretion remained undetectable within the popliteal lymph nodes of all recipients.
Adoptive transfer of Ag-pulsed DCs can enhance protective immunity to T. spiralis

To determine whether these DCs could act as a cellular vaccine against T. spiralis infection, both WT and IL-9t cells were pulsed for 8 h with parasite Ag. Recipient mice were injected with 5 × 10⁵ pulsed or unpulsed BMDCs derived from WT and IL-9t mice 15 days before infection with T. spiralis. Worm burden was assessed on day 8 p.i. (Fig. 5A). Infected controls and recipients of unpulsed WT and IL-9t BMDCs retained a full worm burden 8 days p.i. (data not shown). In contrast, recipient mice of pulsed BMDCs had a significant reduction in the number of worms recovered from the small intestine. Furthermore, IL-9t BMDC recipients demonstrated enhanced expulsion of T. spiralis from the gut. To ensure that this effect was not in part a result of accessory cells within the cellular vaccine, experiments were also undertaken using BMDC preparations that had been subjected to MACS separation before antigenic pulse. These cells also induced enhanced expulsion of T. spiralis from the small intestine 8 days p.i. (Fig. 5B).

Adoptive transfer of WT and IL-9t BMDCs enhances Th2 immune responses within MLN

Whether the difference in worm burden could be accounted for by enhanced type 2 cytokine production within the MLN induced by injection of BMDCs in a time-dependent manner was assessed by restimulation of MLN on days 4 and 8 p.i. (Fig. 6). It is clear that injection of either WT or IL-9t BMDCs into recipient mice enhanced the type 2 cytokines secreted by MLN cells. However, injection of IL-9t BMDCs produced no apparent enhancement over WT BMDCs on day 4 p.i. By day 8 p.i., IL-9t BMDC recipients showed reduced IL-5 production compared with both infected controls and WT BMDC recipients. The levels of IL-4, IL-9, and IL-13 secreted by MLN cells in both BMDC recipients and infected controls had increased to comparable levels, whereas IL-10 secretion was reduced compared with infected controls. Both BMDC recipients showed down-regulation of IL-12 compared with infected controls on day 4 p.i., and by day 8 p.i., IL-12 was undetectable in all infected animals. IFN-γ secretion was abrogated in DC recipients by day 4 p.i. and was absent in all groups by day 8 p.i.

Immunization with IL-9t DCs leads to up-regulation of the parasite-specific IgG1 response that is absent after parasite infection

IL-9t BMDC recipients showed an enhanced level of IgG1 in serum 14 days after injection of cells (Fig. 7). WT BMDC recipients also showed a slight elevation compared with naive mice; however, this was significantly lower than that in IL-9t BMDC recipients. Upon infection with T. spiralis, the level of IgG1 in serum declined in both cell recipient groups. Parasite-specific IgG2a remained undetectable in all groups.

Intestinal mastocytosis is elevated in IL-9t BMDC recipients

IL-9 is known to be a potent inducer of mastocytosis. To determine whether IL-9 derived from BMDCs accelerated worm expulsion by inducing increased levels of mastocytosis, the number of mast cells within villi of the small intestine was counted. The level of
intestinal mastocytosis in recipients of IL-9t BMDCs was significantly elevated compared with WT BMDC recipients and infected controls (Fig. 8).

Discussion

DCs are key cells involved in linking innate and adaptive immune responses. The presentation of Ag and the secretion of both cytokines and chemokines play an integrated role in initiating either Th1 or Th2 immunity. It is well characterized that DCs have the capacity to secrete a variety of cytokines, such as bioactive IL-12, IL-10, IL-27, IL-6, IL-1, TNF-α, and IL-2, when primed with different antigenic stimulations. Few studies have indicated a link between IL-9 and DCs. Human CD83+ blood DCs have been shown to produce IL-9 mRNA 6 h after stimulation with PMA, which was down-regulated by 24 h after stimulation (16). The ability of blood DCs or those isolated from a murine model to actively secrete IL-9 and their potential role in T cell priming have yet to be investigated.

IL-9 is a critical cytokine involved in generating a protective immune response to intestinal nematodes. Previous work from our group has identified a role for IL-9 in the expulsion of both T. spiralis (10) and T. muris (17) from the intestine. Administration of IL-9-secreting hybridoma cell lines before infection with T. muris allowed the level of IL-9 to be increased during infection without raising it to the normal level in IL-9t mice. In these experiments, as in IL-9t mice, expulsion was significantly accelerated, and intestinal mastocytosis was significantly enhanced. This paper demonstrates that BMDCs can be successfully derived from mice genetically manipulated to constitutively secrete large amounts of IL-9. Before antigenic pulse with T. spiralis Ag, WT and IL-9t BMDCs were characterized as CD8α−, CD11c−, CD80low, CD86low, and MHC class IIlow-high expressing cells. Upon antigenic pulse, WT BMDCs up-regulated the expression of both costimulatory molecules CD80 and CD86 as well as MHC class II. In contrast, IL-9t BMDCs up-regulated only CD86 and MHC class II. This expression pattern may account for the enhanced Th2 immune responses after IL-9 secretion. CD86 has been well documented to play an important role in Th2 immune responses, in particular during parasitic infection. In our laboratory and others, CD86 has been demonstrated to have an important protective role against infection with the gastrointestinal dwelling nematode Trichuris muris. In the absence of CD86 signaling, normally resistant strains become susceptible to infection (18). Additional evidence for the role of CD86 in Th2 immunity reported by Brown et al. (19) clearly showed that the absence of CD86 during

Leishmania infection enhanced parasite clearance with a reduction in Th1 cytokine secretion. More recently, Merle Elloso et al. (20) described the importance of CD86 signaling during initiation of the early immune response to Leishmania infection, showing the critical need for CD86 expression in the induction of IL-4 secretion and T cell proliferation. Furthermore, high expression of CD86 during Ag presentation can induce much higher titers of IL-4 secreted by naive T cells compared with CD80 signal alone (21, 22).

Overall, although this is not the first publication to show differential expression of CD80/CD86 costimulatory molecules on APC populations in response to Th2-inducing Ags (23), it is a novel result of DC expression in response to parasitic gut nematodes and may indeed be an essential marker for enhanced resistance to intestinal nematodes and, in this model, the accelerated expulsion of T. spiralis from the small intestine. The data presented in this study imply that environmental IL-9 can alter the DC phenotype, and in the presence of high titers of the potent Th2 cytokines, DC function to prime naive T cells to a Th2 phenotype with secretion of IL-4 and low levels of IFN-γ.

To determine whether the secretion of IL-9 by BMDCs could have an effect on the types of cytokines secreted in BMDC cultures, cells were stimulated for 24 h with either T. spiralis Ag or LPS. Within 24 h, IL-9t BMDCs secreted higher levels of IL-12 than WT cells. The secretion of IL-12 by DCs is a potent inducer of IFN-γ by T cells in vivo (24). Both CD8α+ and CD8α− DCs have been shown to secrete IL-12, although CD8α− DCs generally secrete lower levels of IL-12 in vitro (25). IL-4 has previously been described to provide a negative feedback mechanism on DCs and induce DCs to produce bioactive IL-12 (26). Because IL-9t
BMDCs appear to secrete higher levels of IL-12 than WT cells, it is conceivable that the secretion of the potent Th2 cytokine IL-9 may induce this increase, as does IL-4, and in doing so accelerates the secretion of IL-12 compared with that by WT BMDCs. As expected, only IL-9t cells secreted detectable levels of IL-9, and neither secreted detectable levels of IL-4.

To investigate whether the secretion of IL-9 by BMDCs could influence the immune response toward a Th2 phenotype, BMDCs were cultured in the presence of naive WT MLN CD4+ T cells. Indeed, in the presence of the manipulated BMDCs, T cell proliferation was enhanced. This proliferation was Ag dependent, because IL-9t BMDCs pulsed with *T. spiralis* Ag showed enhanced T cell stimulatory capacity compared with those pulsed with LPS. The WT BMDC cocultures, in the presence of Th2-or LPS-pulsed cells, showed similar levels of T cell proliferation.

In both WT and IL-9t BMDC cocultures, the Th2 cytokines IL-4, IL-5, IL-9, and IL-13 could be quantified, and there was a distinct trend for IL-9t cultures to contain elevated levels of all cytokines present compared with the WT cultures. Although DCs have been shown to secrete IL-4 under certain environmental conditions (27), they have not been found to secrete IL-5, IL-9, and IL-13. Therefore, it is most likely that T cells within these interactions are the main producers of these cytokines. The major source of IL-9 within IL-9t cultures was likely to be BMDCs themselves; however, the possibility that T cells also produced IL-9 cannot be overlooked, because IL-9 was detectable within WT cell cultures. IL-9 levels were also elevated after antigenic pulse of the IL-9t DCs, thus reflecting Ag-specific immune responses.

It can be concluded, therefore, that the type of Ag used to initially prime DCs can alter the proliferative response of the naive T cell. Furthermore, the ability of DCs to secrete high levels of IL-9 induces a strong Th2 immune response in vitro.

To explore this further, pulsed DCs were injected s.c. into the heel of naive WT recipients. By restimulating the popliteal and inguinal lymph nodes with *T. spiralis* Ag, the direct affect of primed DCs on naive T cells could be identified. In agreement with the in vitro data, both WT and IL-9t DCs induced the secretion of IL-4 and IL-9 from both types of lymph node cells in vivo, although more prominently in the draining popliteal lymph node. As seen in vitro, low levels of IL-9 could be detected in lymph node cultures from WT BMDC recipients and, although not conclusive, were most likely to originate from the in situ-primed T cells. Therefore, some of the IL-9 detected in the cell supernatant from IL-9t BMDC recipients could in part be the result of T cell stimulation. Of particular note is the down-regulation of IFN-γ seen within the popliteal lymph node cells from the IL-9t BMDC recipients, and this occurs in the presence of higher levels of Th2 cytokines. It is possible that the lack of Th1 cytokines in combination with the magnitude of the Th2 cytokine response is important in the disease outcome.

Because IL-9t BMDCs have the ability to induce T cell proliferation in a Th2-dependent manner, experiments were conducted to determine whether adoptive transfer of BMDCs could potentially vaccinate mice against infection with *T. spiralis* and induce accelerated expulsion of adult worms from the small intestine. WT BMDC-vaccinated mice showed enhanced expulsion of adult worms from the small intestine, elevated parasite-specific IgG1 secretion, and a significant difference from the infected controls in intestinal mastocytosis, although no difference in the secretion of type 2 cytokines after restimulation of MLN cells was apparent at the time points examined. The injection of IL-9t DCs into normal WT recipients initiated a cascade of events leading to an accelerated expulsion of *T. spiralis* from the small intestine with up-regulation of parasite-specific IgG1 compared with WT cell recipients. This increase in specific IgG1 is in agreement with other published work, which showed an elevation in Ab responses after injection of pulsed DCs (3–6). Furthermore, IL-9 is known to elevate IgG1 levels in serum (28, 29). The parasite-specific IgG1 level was abolished after infection. Possible reasons for this may be that upon reinfection, the Ab is absorbed out by the large quantity of incoming Ag secreted by the parasite or that IgG binds to the IgG receptors on cells such as mast cells (30), which are induced after infection.

Injection of BMDCs led to the enhanced secretion of IL-4, IL-5, IL-9, and IL-13 within the MLN 4 days p.i. with *T. spiralis*, followed by a reduction in detectable IL-5 in IL-9t BMDC recipients on day 8 p.i. This reduction in IL-5 may be due to the accelerated worm expulsion, and therefore, by day 8 the MLN response was beginning to diminish. Interestingly, both WT and IL-9t recipients

![FIGURE 7. Level of IgG1 in the serum on days −1 and 8 p.i. IL-9t or WT BMDCs were pulsed for 8 h with *T. spiralis* Ag. Cells (0.5 × 10^{6}) were adoptively transferred (i.v.) into WT recipients. Fifteen days after transfer, recipients were infected with 200 *T. spiralis* larvae. Recipients were bled (i.v.) 14 days after transfer (1 day before infection) and on day 8 p.i. (cardiac puncture). A, Level of IgG1 on day 14 after transfer; B, level of IgG1 8 days p.i. Values are the mean OD value for each group at a 1/40 dilution ± SEM. * Significantly different from infected control; **, significantly different between WT and IL-9t recipients (p < 0.05). Data are representative of five individual experiments.](http://www.jimmunol.org/)

![FIGURE 8. Number of mast cells within the villi of the small intestine 8 days p.i. IL-9t or WT BMDCs were pulsed for 8 h with *T. spiralis* Ag. Cells (0.5 × 10^{6}) were adoptively transferred (i.v.) into WT recipients (five mice per group). Fifteen days after transfer, recipients were infected with 200 *T. spiralis* larvae. Gut snips were taken on day 8 p.i., and the number of mast cells was enumerated. Values represent the number of mast cells per 20 villi crypt units (VCU) ± SEM. * Significantly different from infected controls and WT recipients (p < 0.05). Data are representative of five individual experiments.](http://www.jimmunol.org/)
show an abrogation of Th1 cytokine secretion (IL-12 and IFN-γ) early in infection; thus, it may be that it is the lack of Th1 cytokines that is important in disease outcome rather than the magnitude of the Th2 cytokine response.

Infection with T. spiralis is well characterized as an inducer of mastocytosis within the small intestine (31), and expulsion of the parasite from the intestine is highly dependent on the mast cell responses tightly controlled by CD4+ T cells and, in particular, through the secretion of IL-9 (10, 32). Mast cells have more recently been described as crucial cells in the induction of mucosal permeability through active secretion of the serine protease mouse MCP-1; it is through this mechanism that effective expulsion of the parasite is believed to occur (33). In the absence of mast cells, expulsion of T. spiralis from the intestine is severely delayed, most likely through reduced host defense mechanisms such as decreased permeability.

Upon transfer of IL-9 BMDCs, recipient mice demonstrated an elevated level of intestinal mastocytosis. Although injected BMDCs could not be visualized within the small intestine (data not shown), it cannot be conclusively ruled out that BMDCs directly induce mastocytosis within the intestine. However, it is most likely that upon transfer, BMDCs in the presence of IL-9 enhance the induction of intestinal mastocytosis. Although injected BM-DCs could not be visualized within the small intestine (data not shown), it cannot be conclusively ruled out that BMDCs directly induce mastocytosis within the intestine. However, it is most likely that upon transfer, BMDCs in the presence of IL-9 enhance the Th2-induced immune response and lead to a stronger mast cell response. Thus, although no significant enhancement of Th2 immune responses could be seen in MLN after transfer, it is feasible that accelerated/enhanced numbers of activated T cells could move to the site of infection, enhance the observed mast cell hyperplasia, and consequently lead to accelerated expulsion of T. spiralis from the small intestine.

In conclusion, it can be clearly seen that BMDCs pulsed ex vivo with parasite Ags can confer resistance to infection with intestinal helminths and that the manipulation of IL-9 secretion early on in the priming of naive T cells can enhance Th2-mediated immune responses. Thus, IL-9 indeed acts as a potent type 2 adjuvant.

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Disclosures
The authors have no financial conflict of interest.

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